Fluorescence of chlorophyll as a tool to assess the degree of eutrophication of aquatic systems

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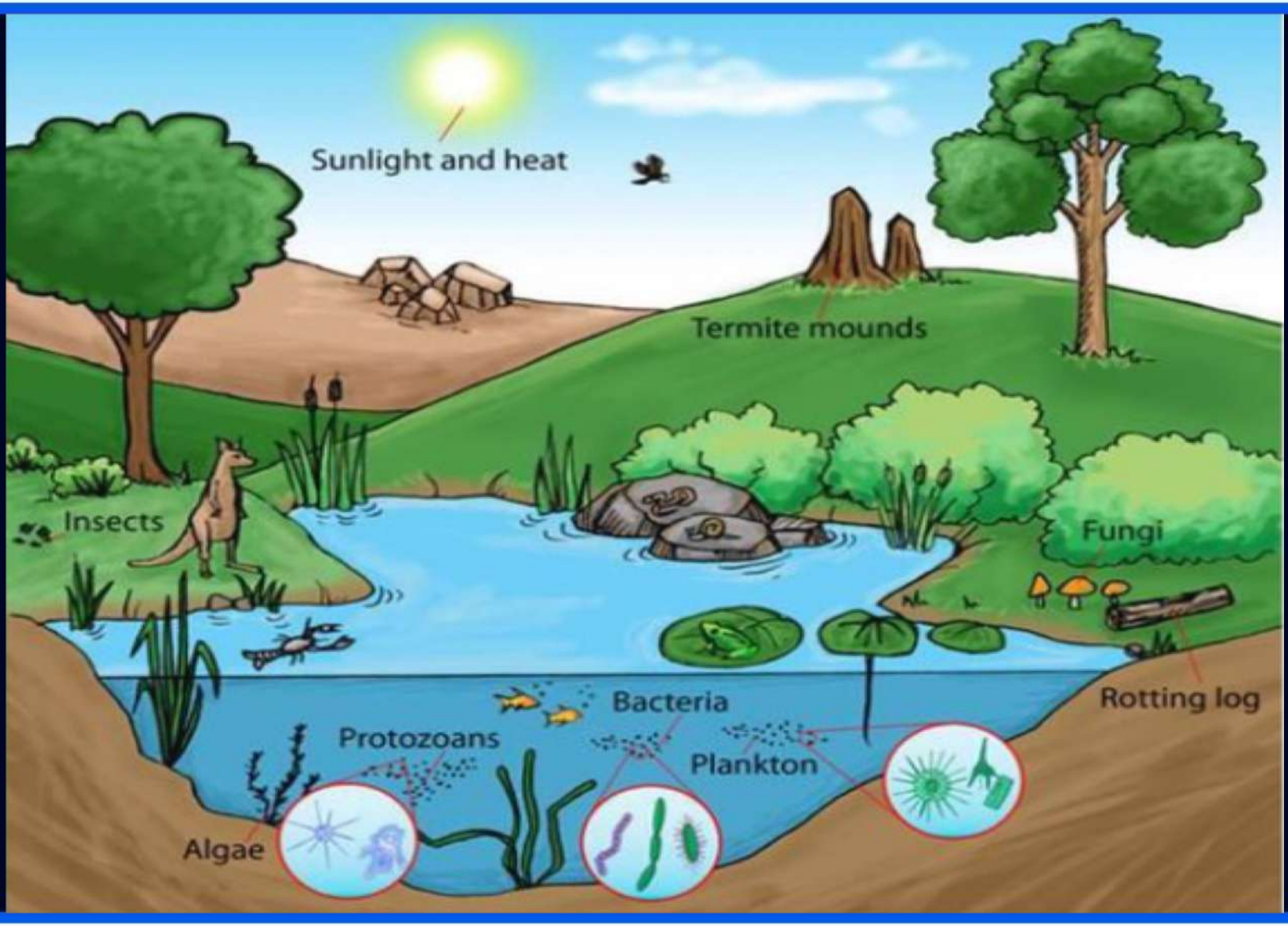
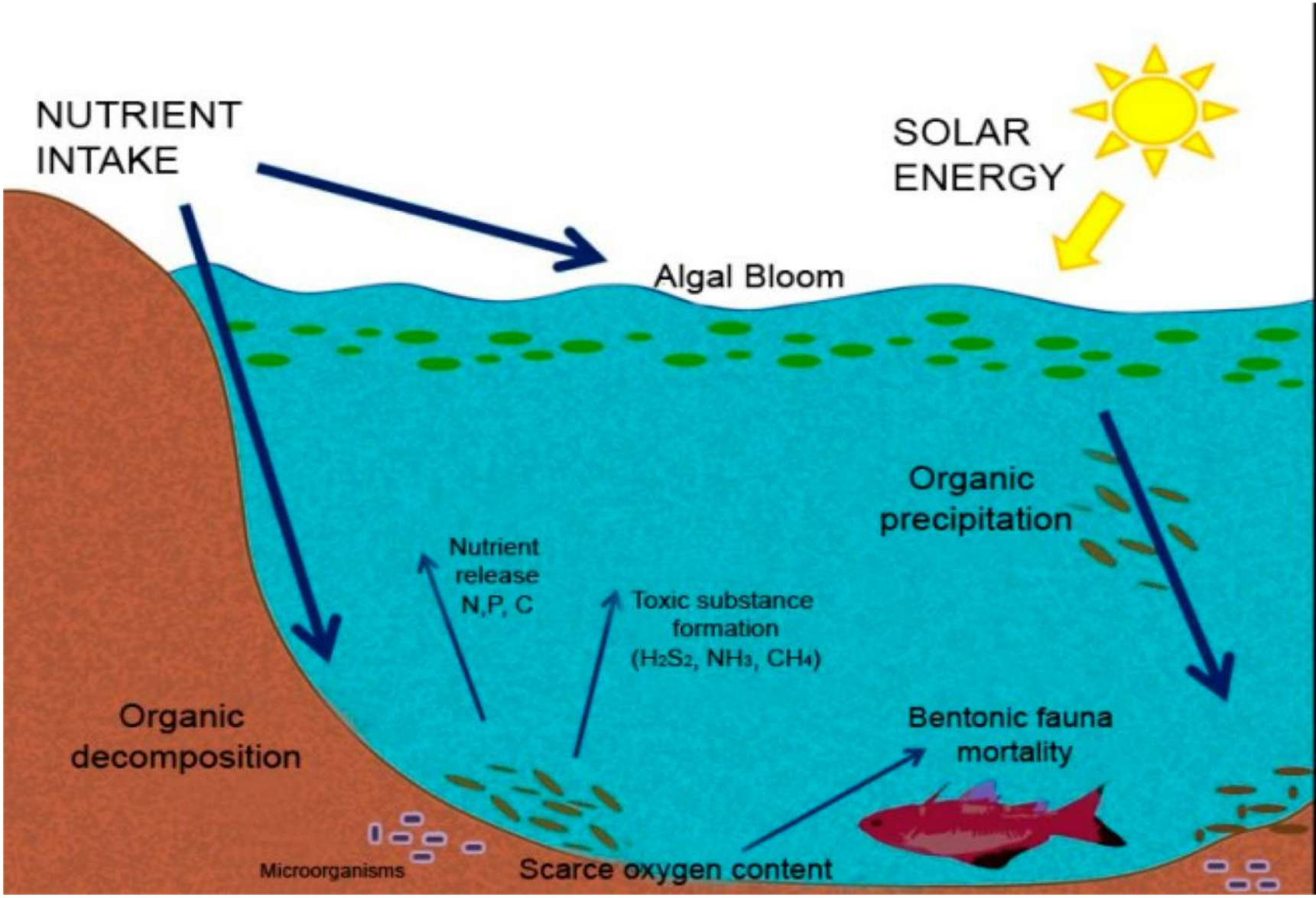
Warsaw University of Life Sciences - SGGW

Ecosystem components and interactions

Anis Webster- https://slideplayer.com/slide/13585533/



Eutrophication, or hypertrophication, is when a body of water becomes overly enriched with minerals and nutrients which induce excessive growth of algae. This process may result in oxygen depletion of the water body.



Eutrophication process representation (Feem re-elaboration from Arpa Umbria, 2009)

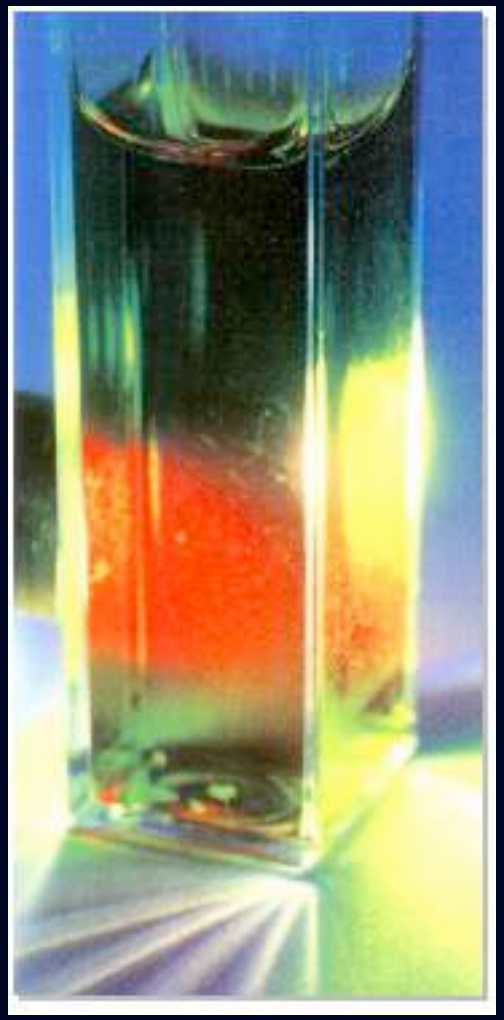
Types of Eutrophication

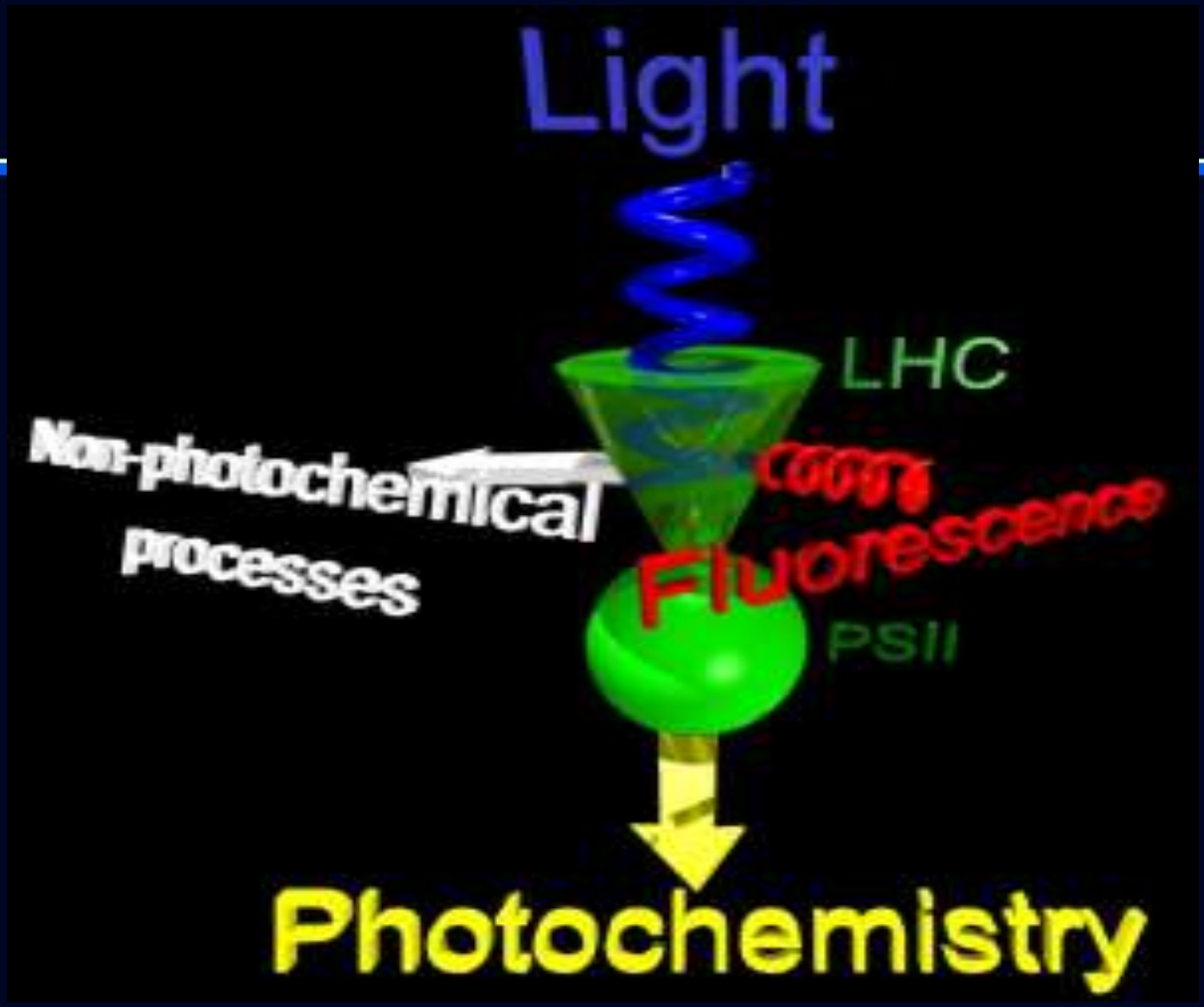
- Natural Eutrophication -- a process that occurs as a lake or river ages over a period of hundreds or thousands of years.
- Cultural Eutrophication -- a process that occurs when humans release excessive amounts of nutrients; it shortens the rate of aging to decades.

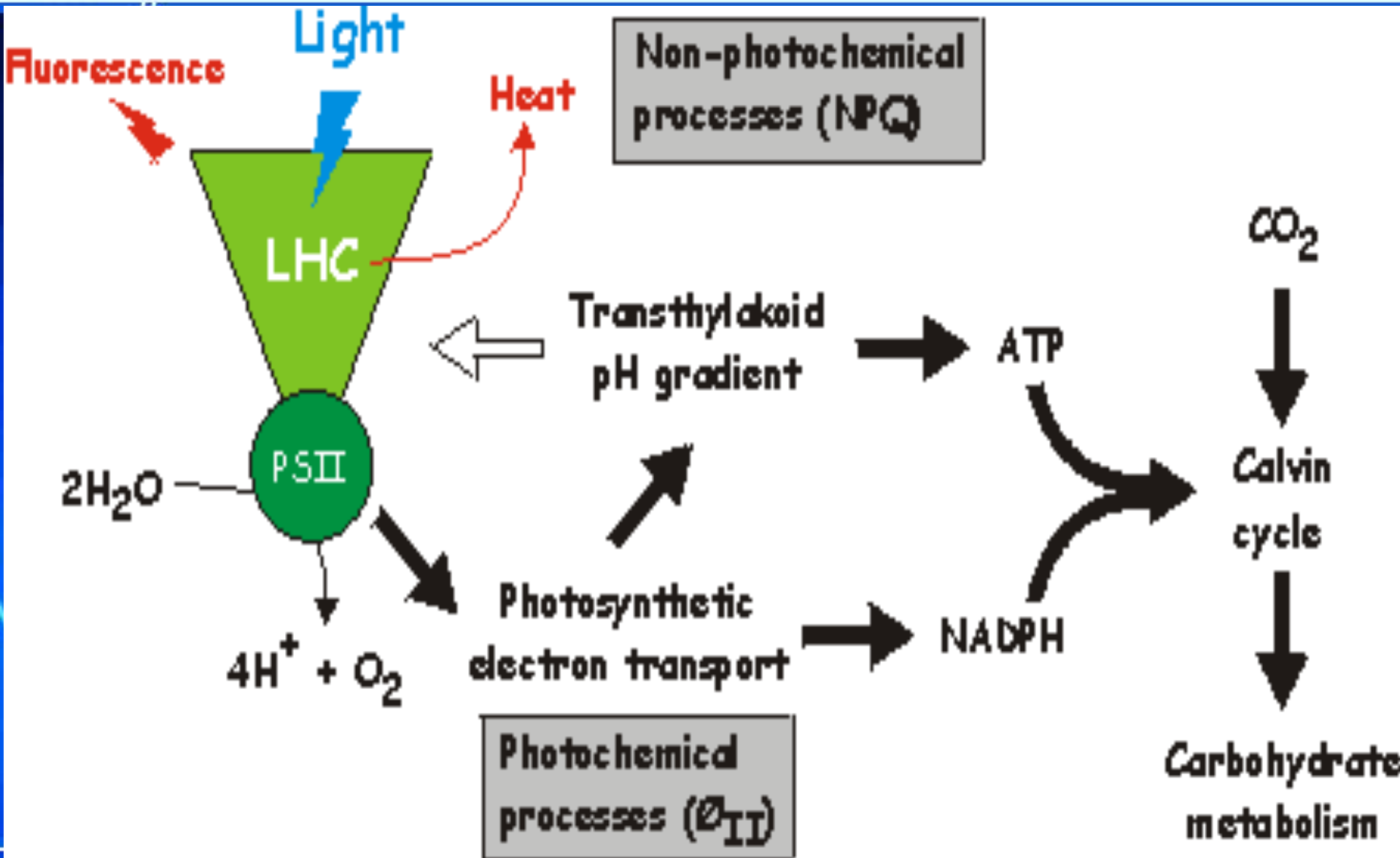
Algal bloom in 2010 along the coast of Qingdao, eastern China (nationalgeographic.it/)

Need for non-invasive and fast methods NOT ONLY to monitor water ecosystems but…..

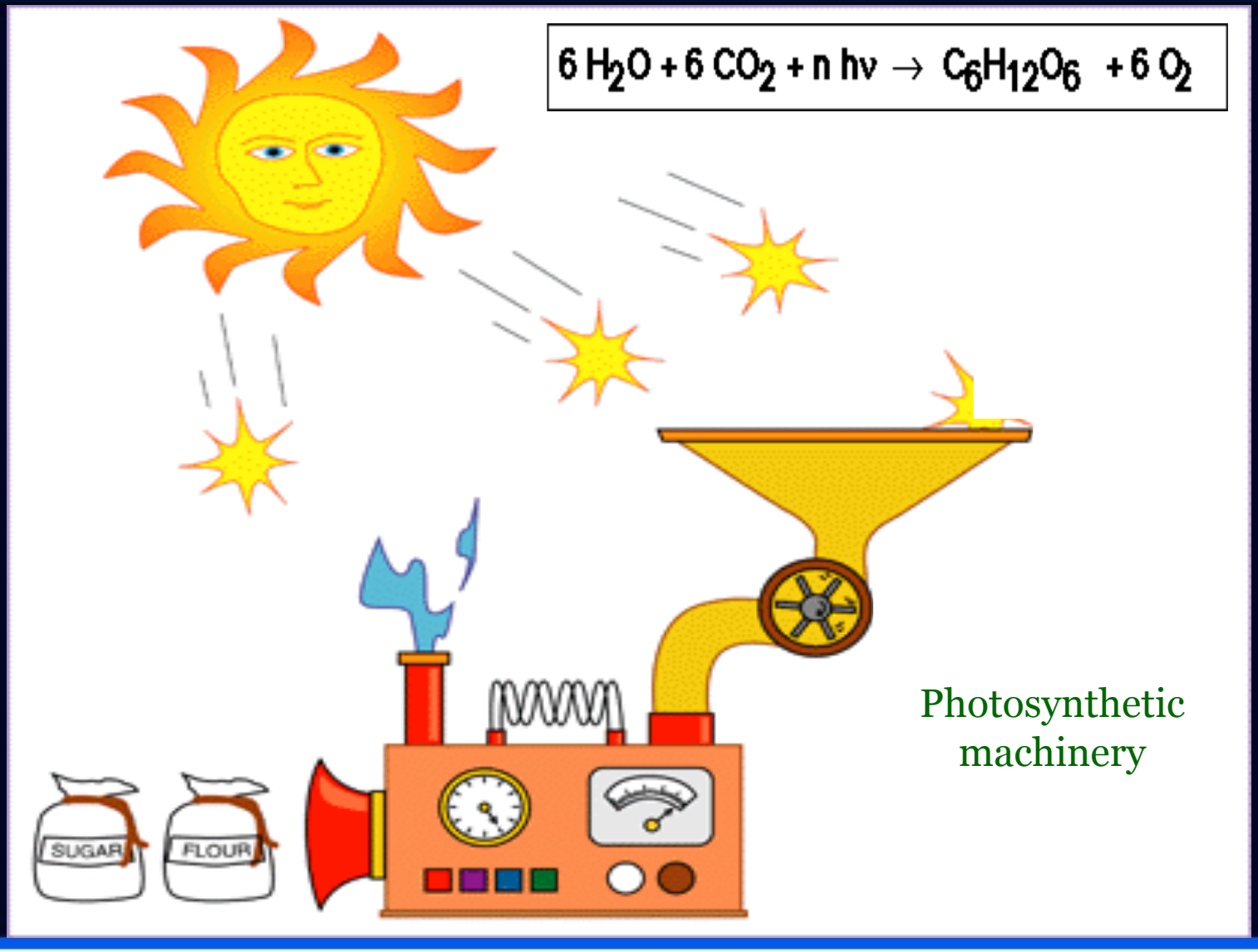
Fluorescencja Chlorofilu a



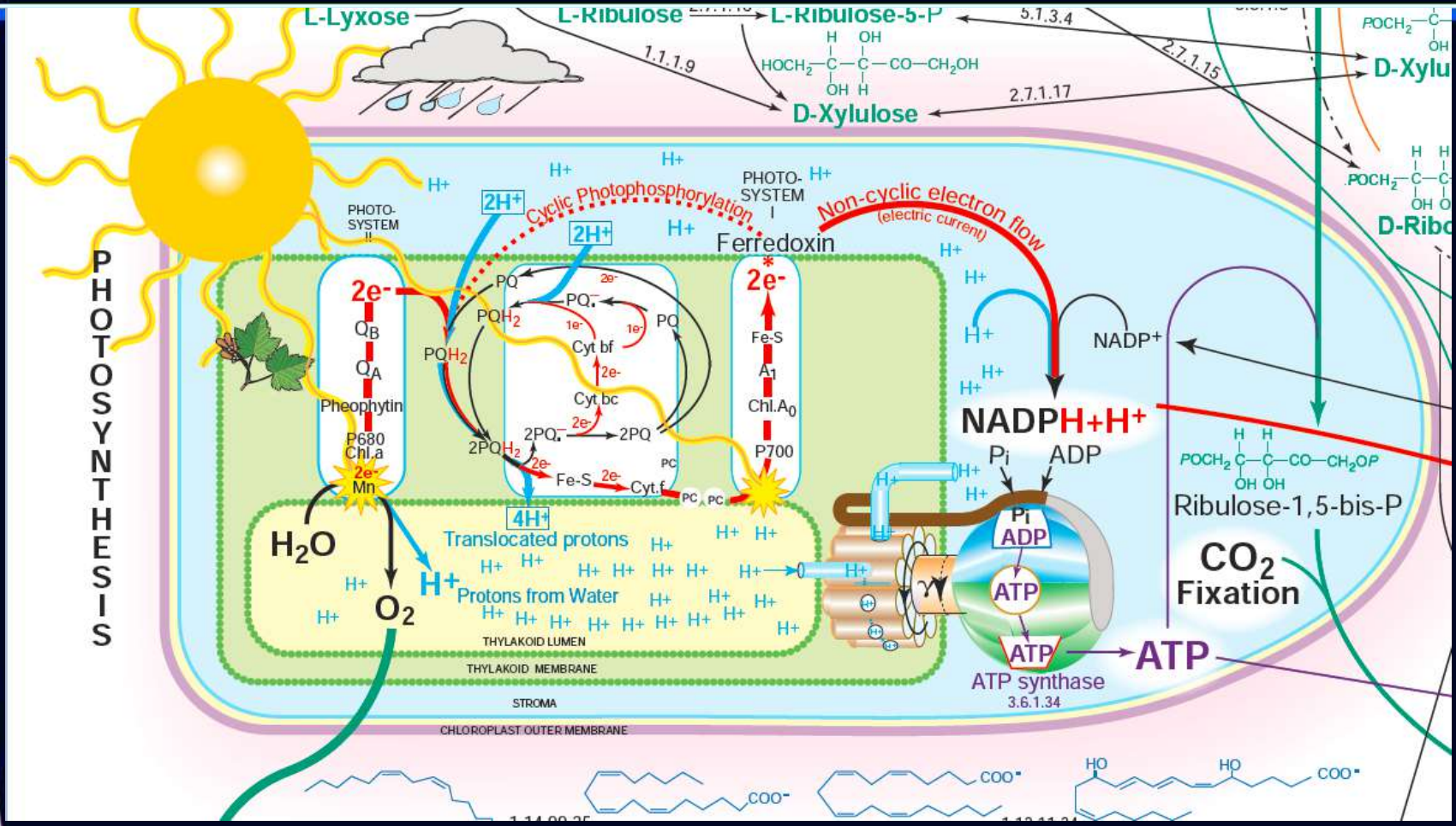

Light
LHC
Non-photochemical processes
Fluorescence
PSII
Photochemistry


Light
Fluorescence
Heat
Non-photochemical processes (NPQ)
LHC
PSII
$2H_2O$
$4H^+ + O_2$
Transthylakoid pH gradient
Photosynthetic electron transport
ATP
NADPH
CO_2
Calvin cycle
Carbohydrate metabolism
Photochemical processes (Φ_{II})

http://bioenergy.asu.edu/photosyn/education/experiments/protein_exp/rcreq4.htm

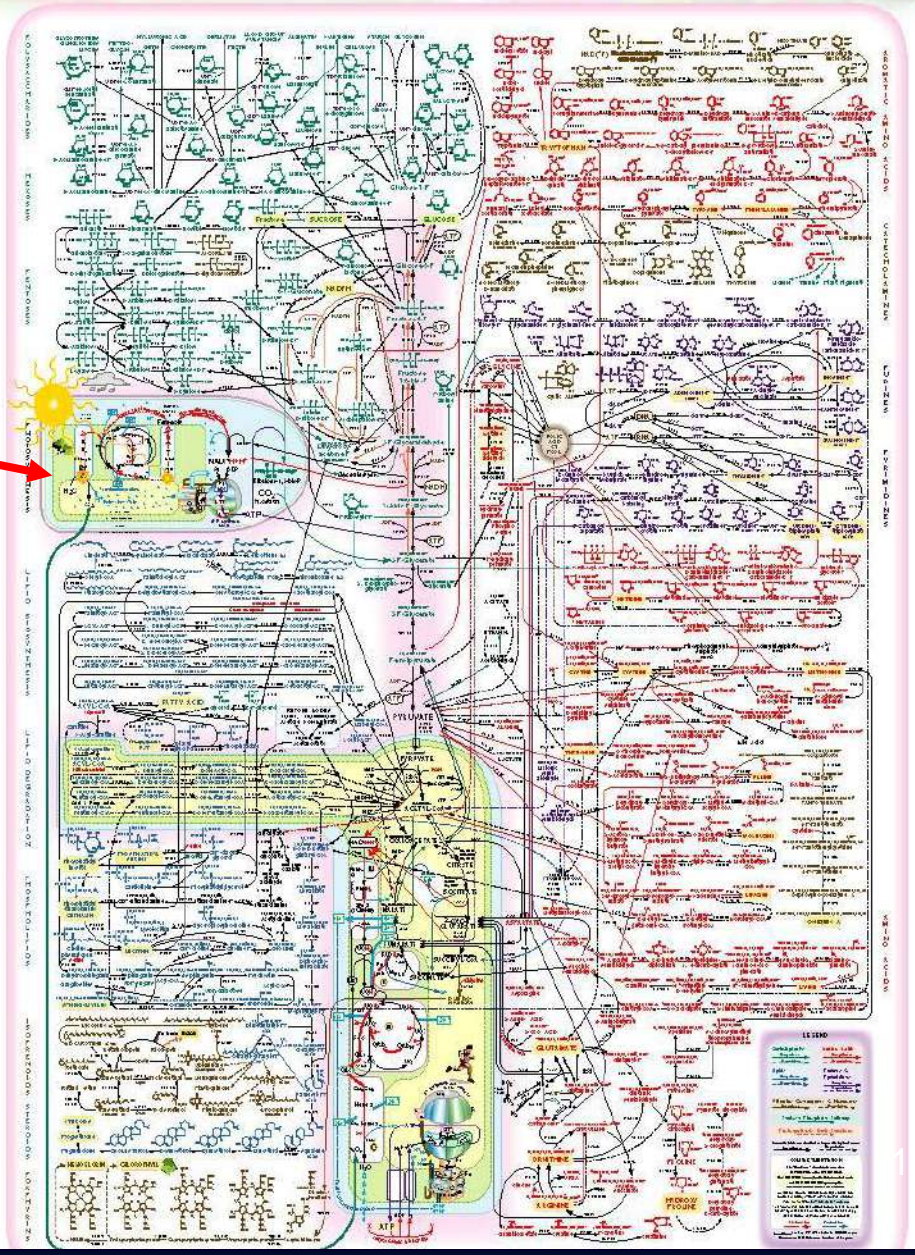


Photosynthetic machinery

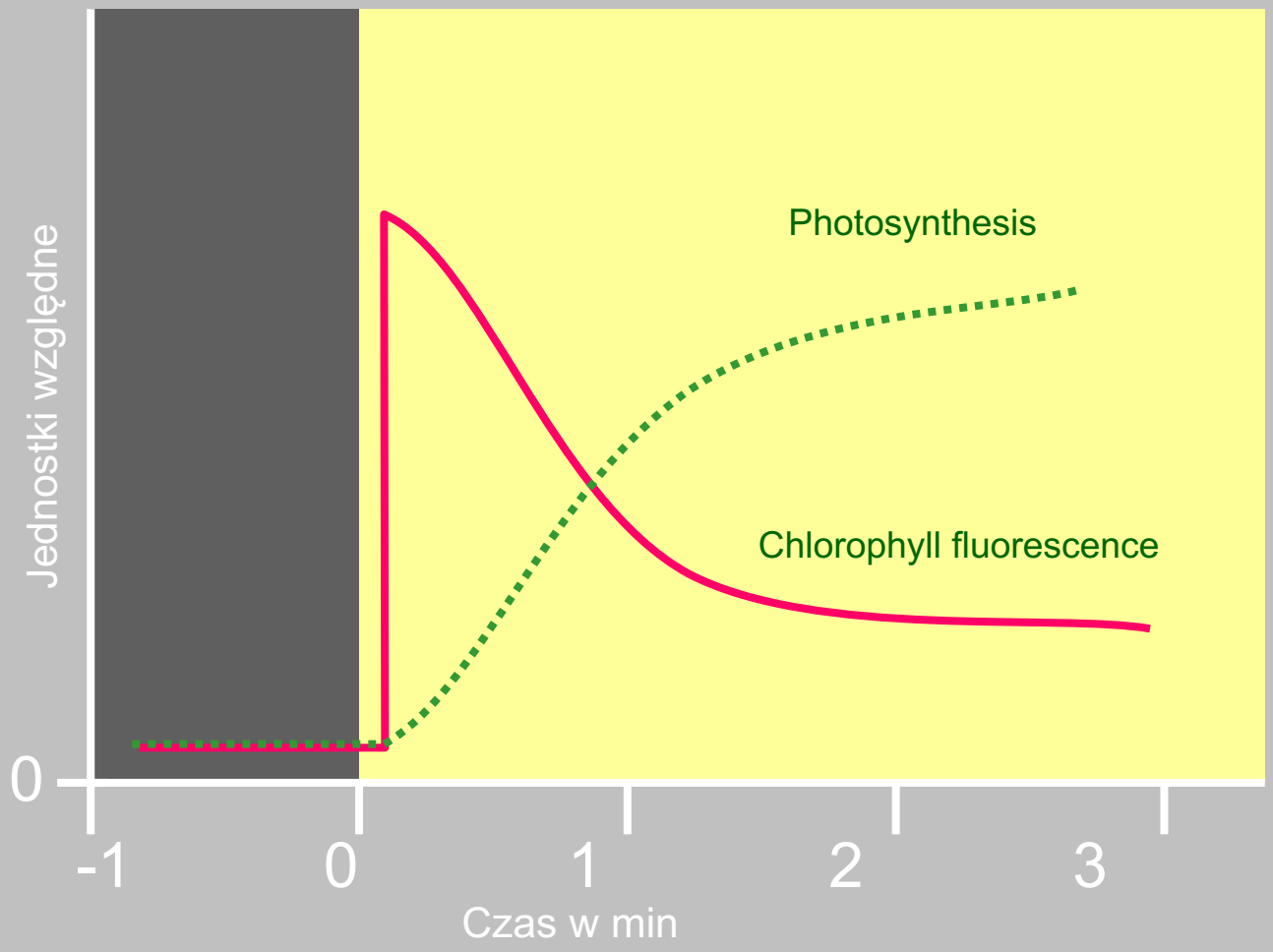

L-Lyxose
L-Ribulose
L-Ribulose-5-P
D-Xylulose
D-Xylu
D-Ribo
1.1.1.9
2.7.1.17
2.7.1.15
5.1.3.4
PHOTOSYNTHESIS
PHOTO-SYSTEM II
PHOTO-SYSTEM I
Cyclic Photophosphorylation
Non-cyclic electron flow
(electric current)
Ferredoxin
2H+
2e-
Q_B
Q_A
Pheophytin
P680
Chl.a
Mn
PQ
PQH_2
Cyt bf
Cyt bc
Fe-S
Cyt.f
PC
A_1
$Chl.A_0$
P700
H_2O
O_2
H+
4H+
Translocated protons
Protons from Water
THYLAKOID LUMEN
THYLAKOID MEMBRANE
STROMA
CHLOROPLAST OUTER MEMBRANE
$NADP^+$
NADPH+H+
Pi
ADP
ATP
ATP synthase
3.6.1.34
Ribulose-1,5-bis-P
CO_2 Fixation

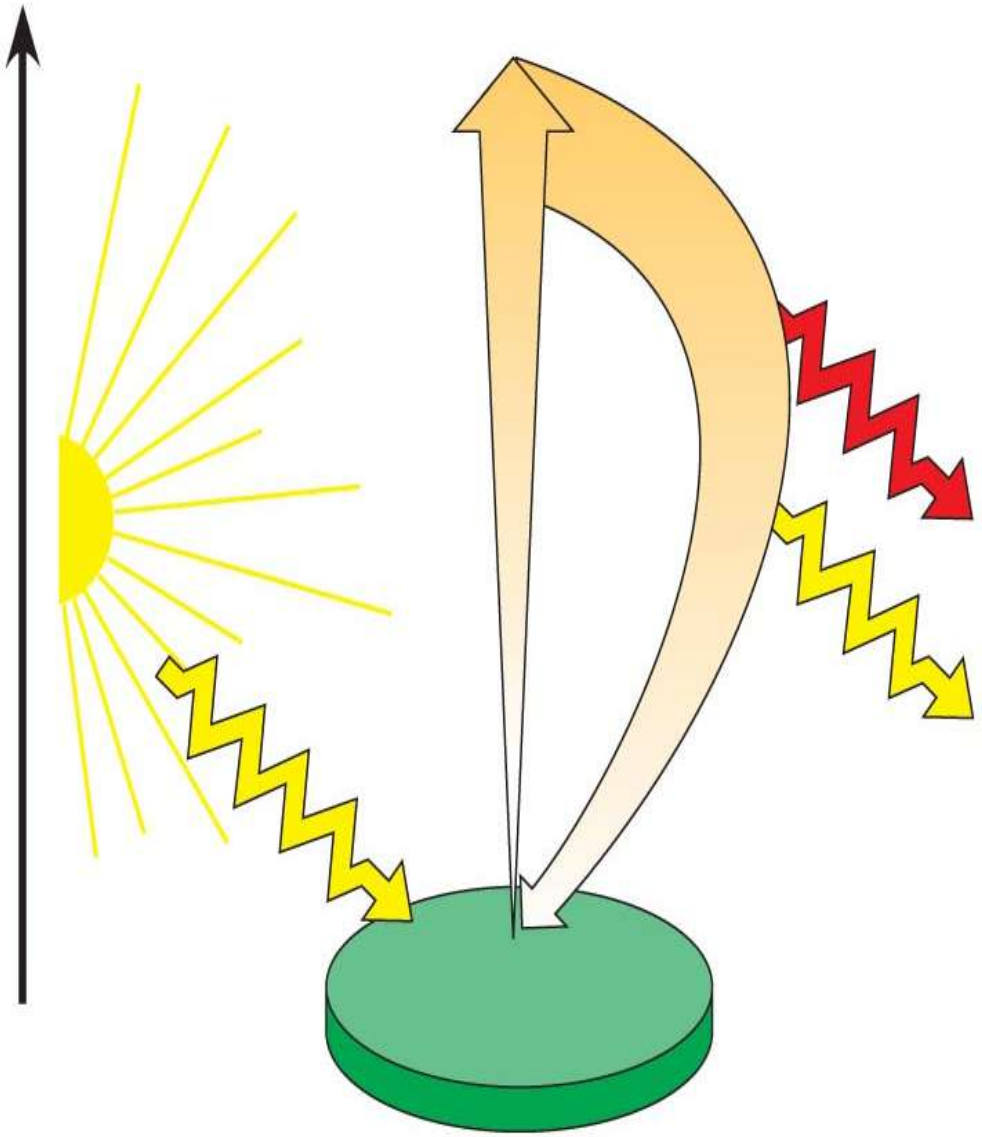
Metabolic Pathways

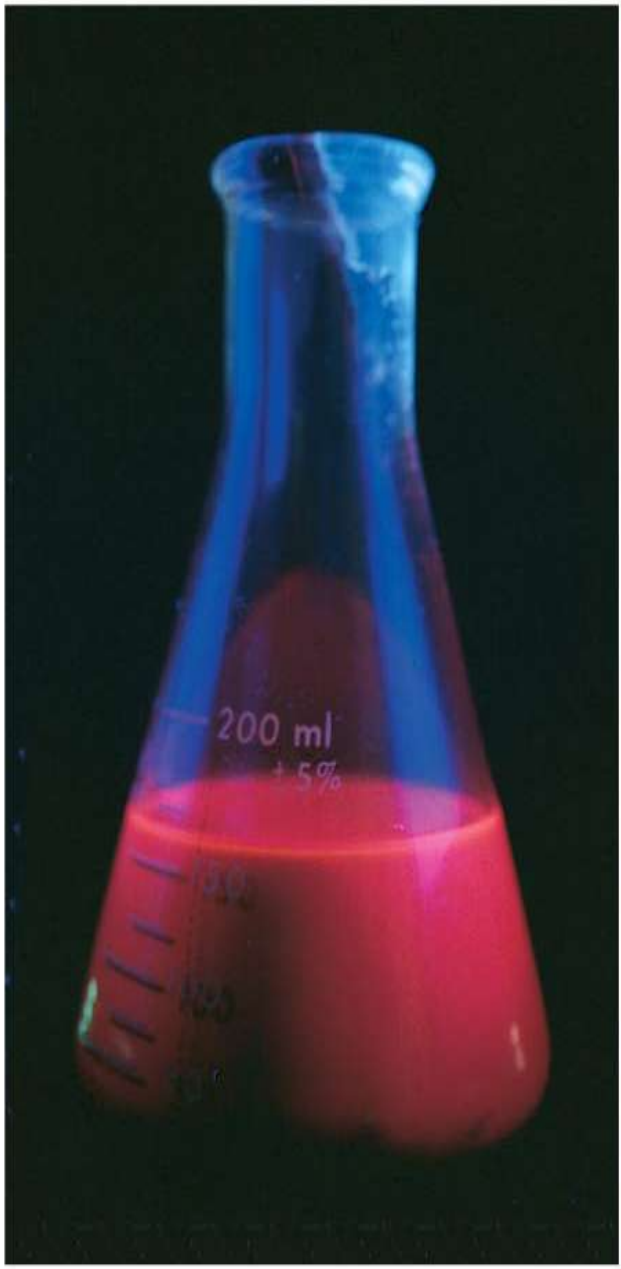
Photosynthesis



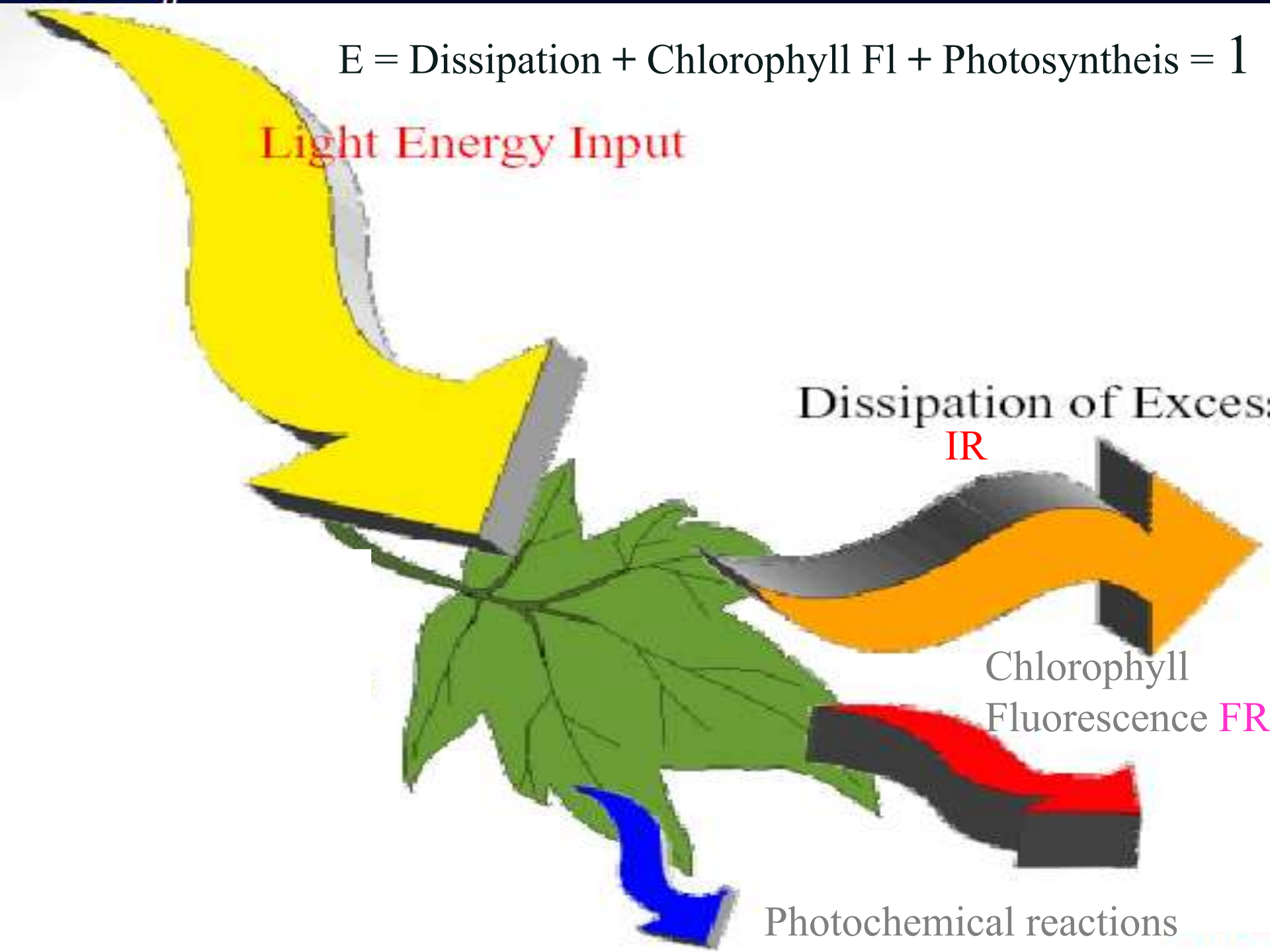
Kautsky Effect







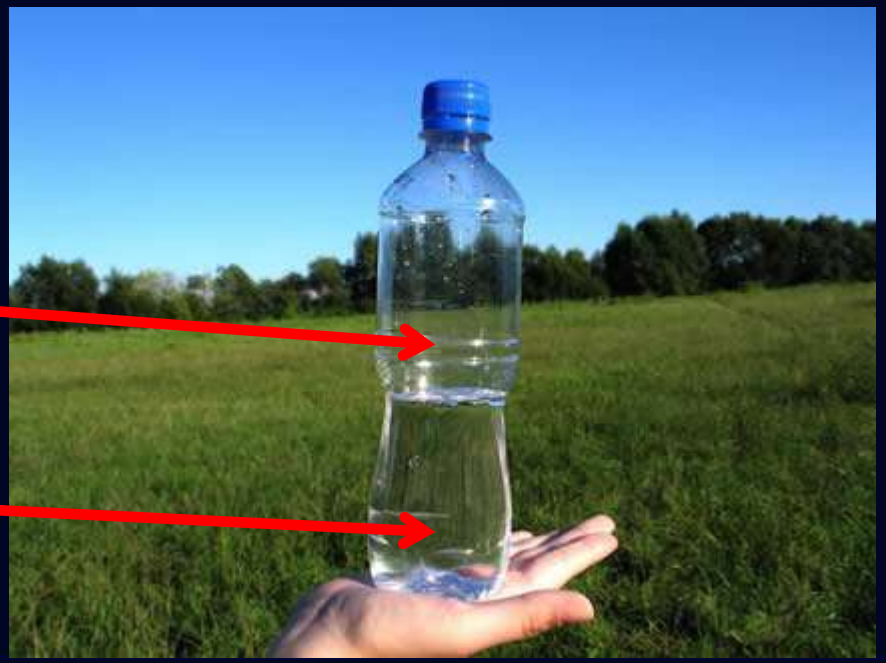
E = Dissipation + Chlorophyll Fl + Photosyntheis = 1



100%

Photosynthesis

Fluorescence
+
Heat



Fluorimeters (stress meters)

After dark adaptation

Fo - fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidised.

Fm - fluorescence level when Qa is transiently fully reduced.

Fv - variable fluorescence (Fm-Fo).

Fv/Fm - maximum quantum efficiency of photosystem II.

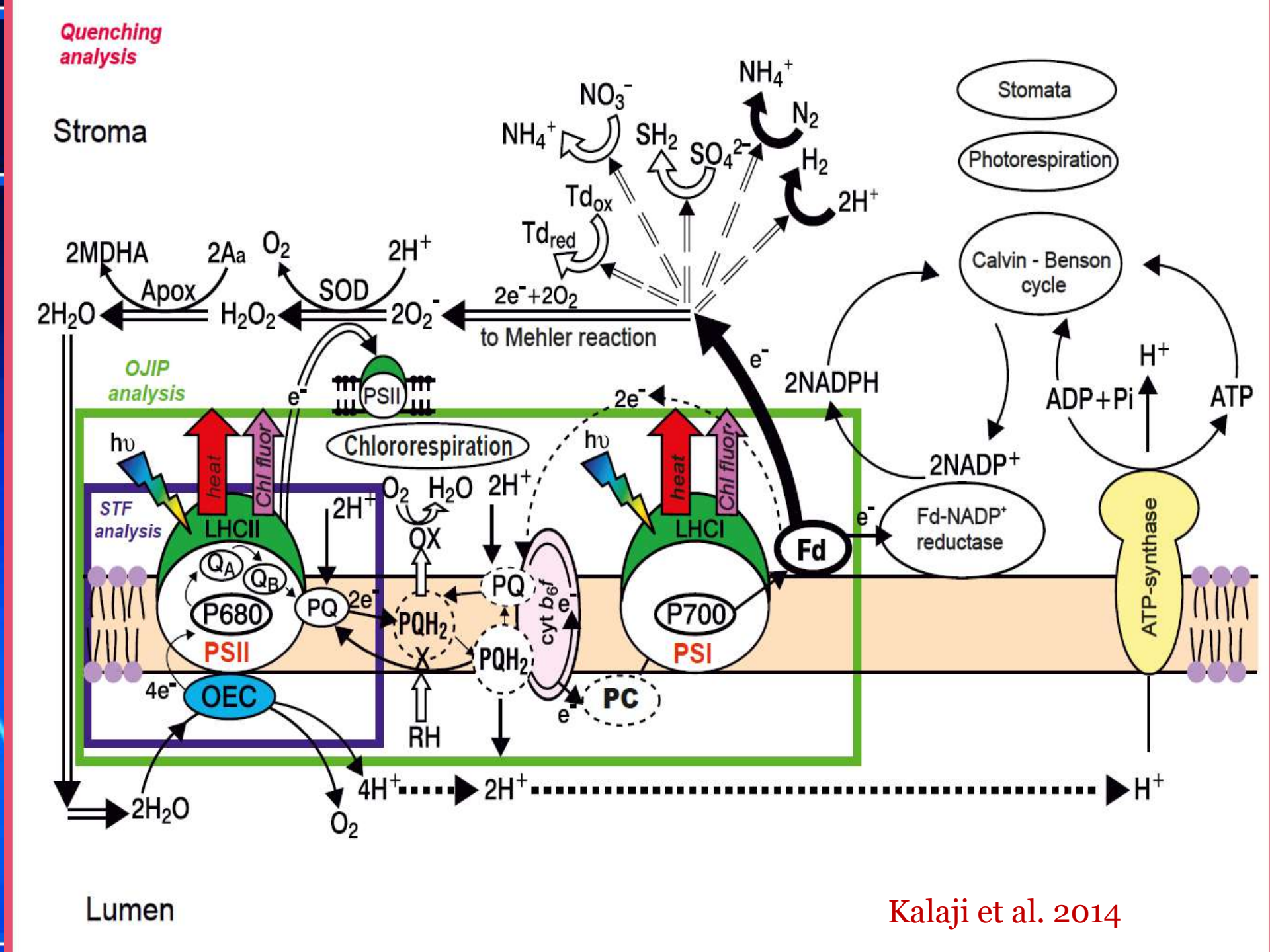
Tfm - time at which Fm occurs.

Area - area over the curve between Fo and Fm, relates to the pool size of PSII electron transport acceptors.

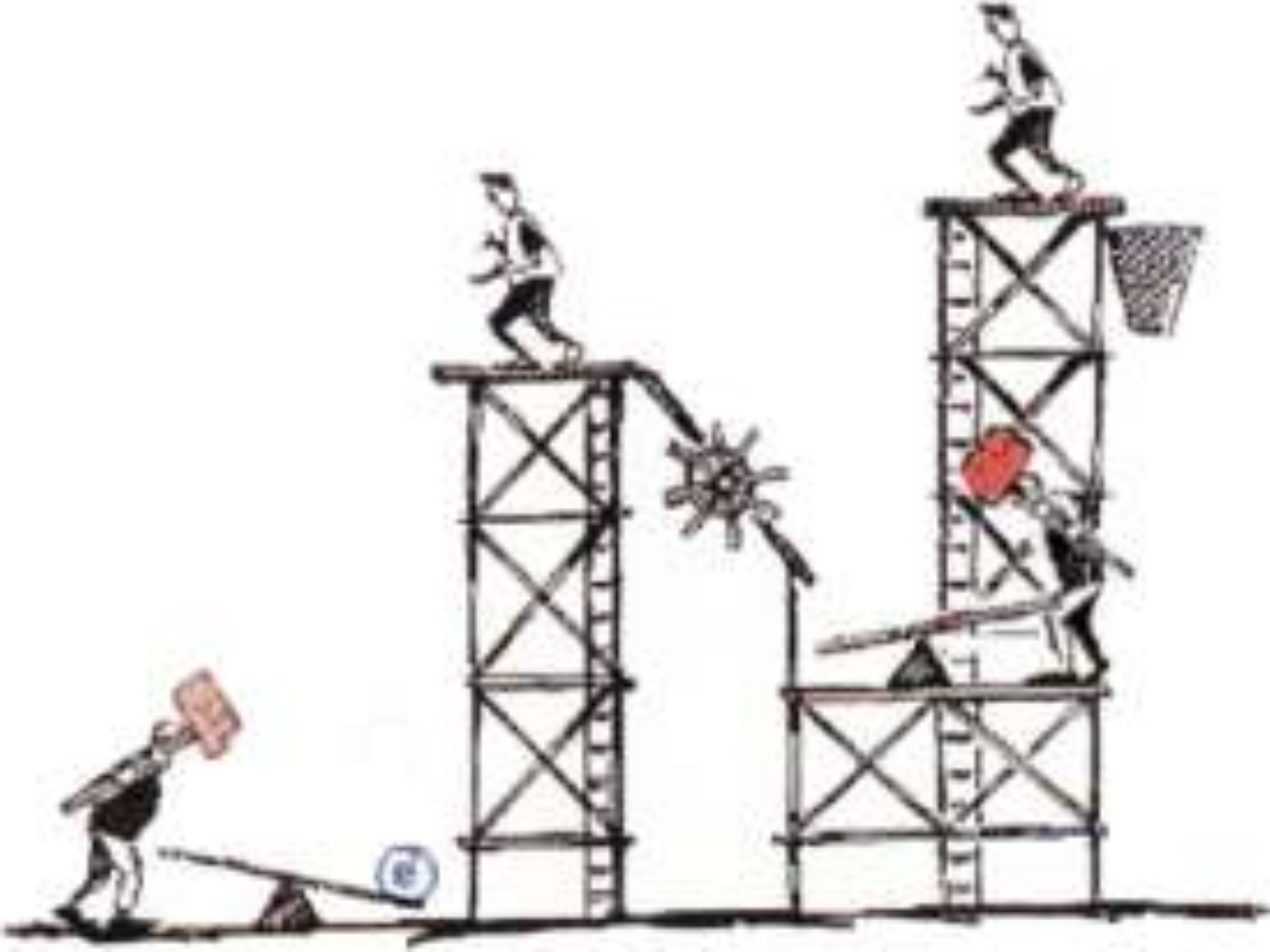
OJIP analysis (Strasser R.J., Srivasatava A. and Govindjee,1995 Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria, Photochemistry and Photobiology, 61, 32-34.).

After light adaptation

Parameter:	Measurement:	Units:	Derivation:
PAR	Incident photosynthetically active radiation	$\mu molm^{-2}s^{-1}$	
Temp	Temperature	°C	
Fs	Steady state fluorescence yield	Bits	
Fm'	light-adapted fluorescence maximum	Bits	
Fv'	Light-adapted variable fluorescence	Bits	= Fm'-Fo'
Fv'/Fm'	Antennae efficiency of PSII	No units	= (Fm'-Fo') / Fm'
ϕ_{PSIIR}	quantum efficiency of PSII	No units	= (Fm'-Fs) / Fm' (Genty *et al.* 1989)
qP	photochemical quenching co-efficient	No units	= (Fm'-Fs) / (Fm'- Fo')
qNP	Non-photochemical quenching co-efficient	No units	= (Fm-Fm') / (Fm-Fo')
NPQ	Alternative definition of non-photochemical quenching	No units	= (Fm-Fm') / Fm'
ETR	Electron Transport Rate	No units	= PAR * 0.5 * 0.84 * ϕ_{PSII}



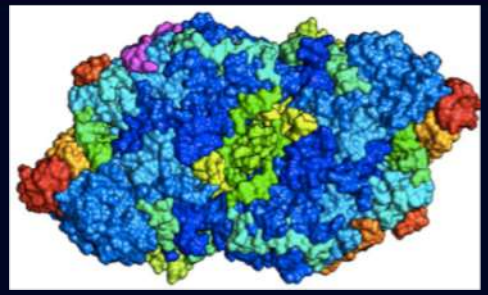
Kalaji et al. 2014



Pulse = 60-80/minute

Maximal quantum yield
Fv/Fm = 0.83-0.85 r.u.

Heart

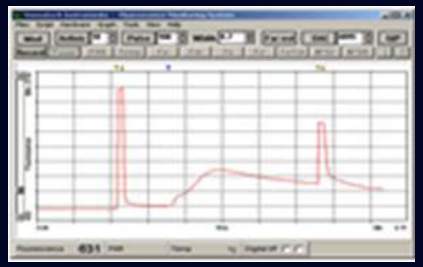


PSII

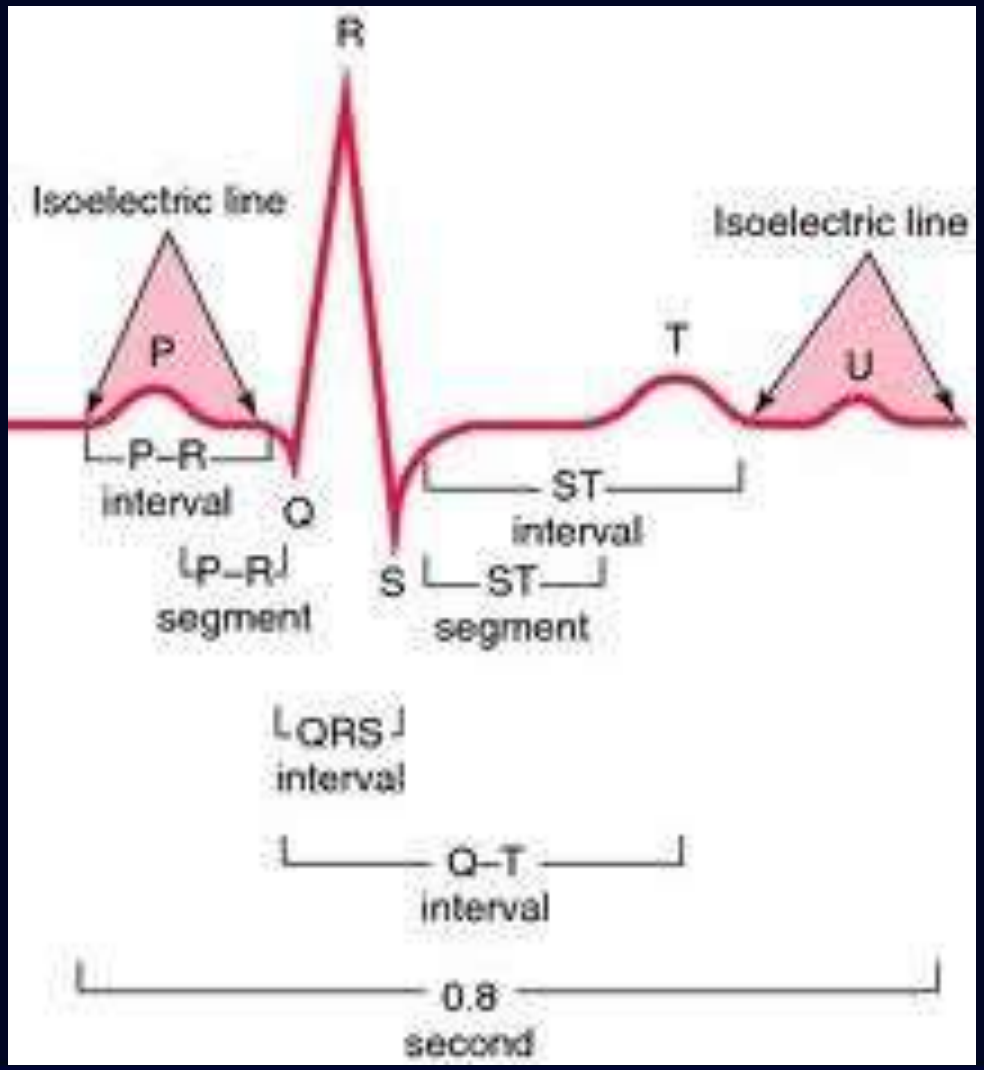
Stethoscope

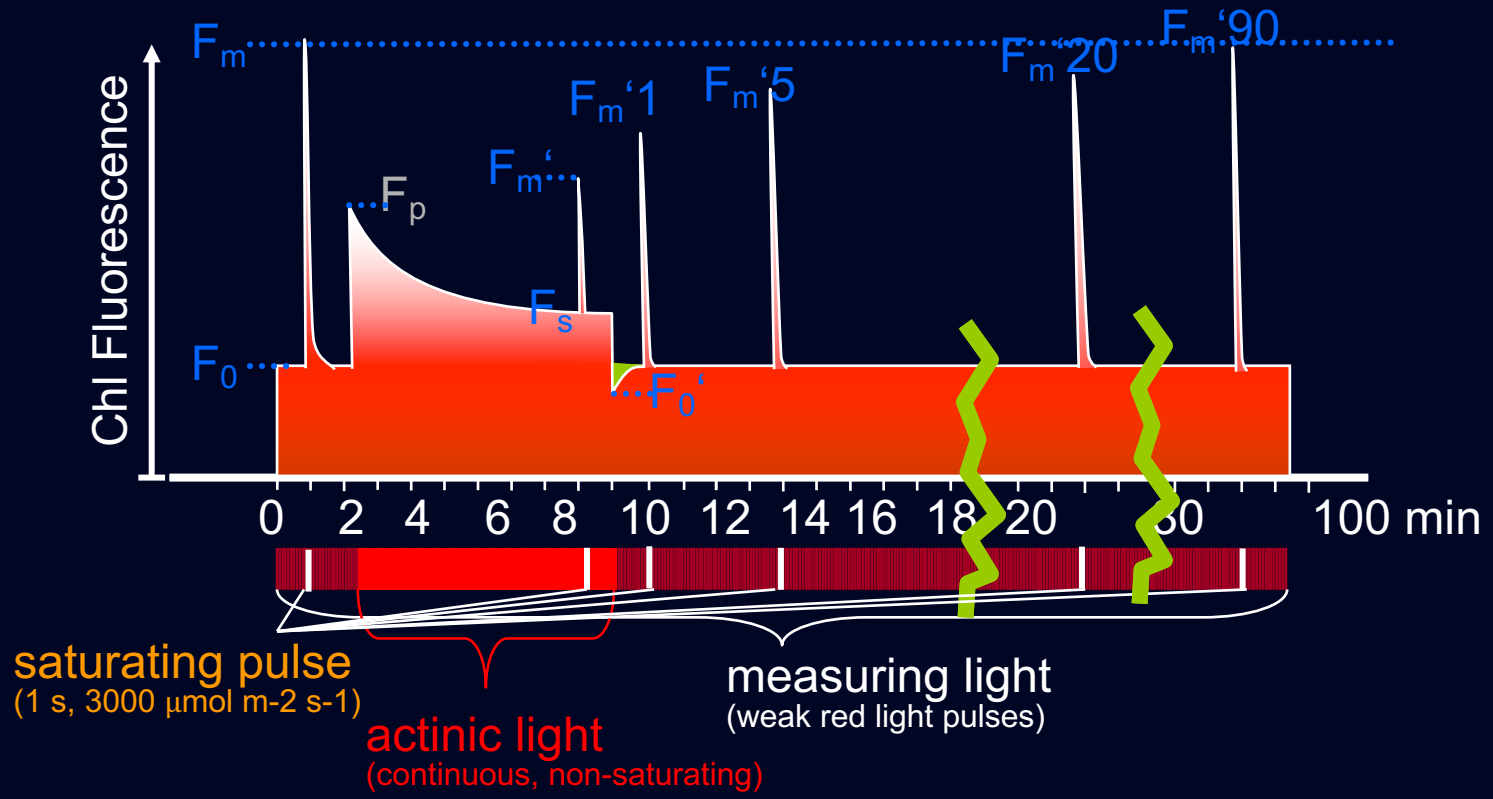
Fluorimeter

Electrocardiograph



Chlorophyll fluorescence induction curve


R
Isoelectric line
Isoelectric line
P
T
U
P–R
interval
Q
ST
interval
P–R
segment
S
ST
segment
QRS
interval
Q–T
interval
0.8
second


Chl Fluorescence
F_m
F_m'90
F_m'20
F_m'1
F_m'5
F_m'
F_p
F_s
F_0
F_0'
0 2 4 6 8 10 12 14 16 18 20 30 100 min
saturating pulse
(1 s, 3000 µmol m-2 s-1)
actinic light
(continuous, non-saturating)
measuring light
(weak red light pulses)

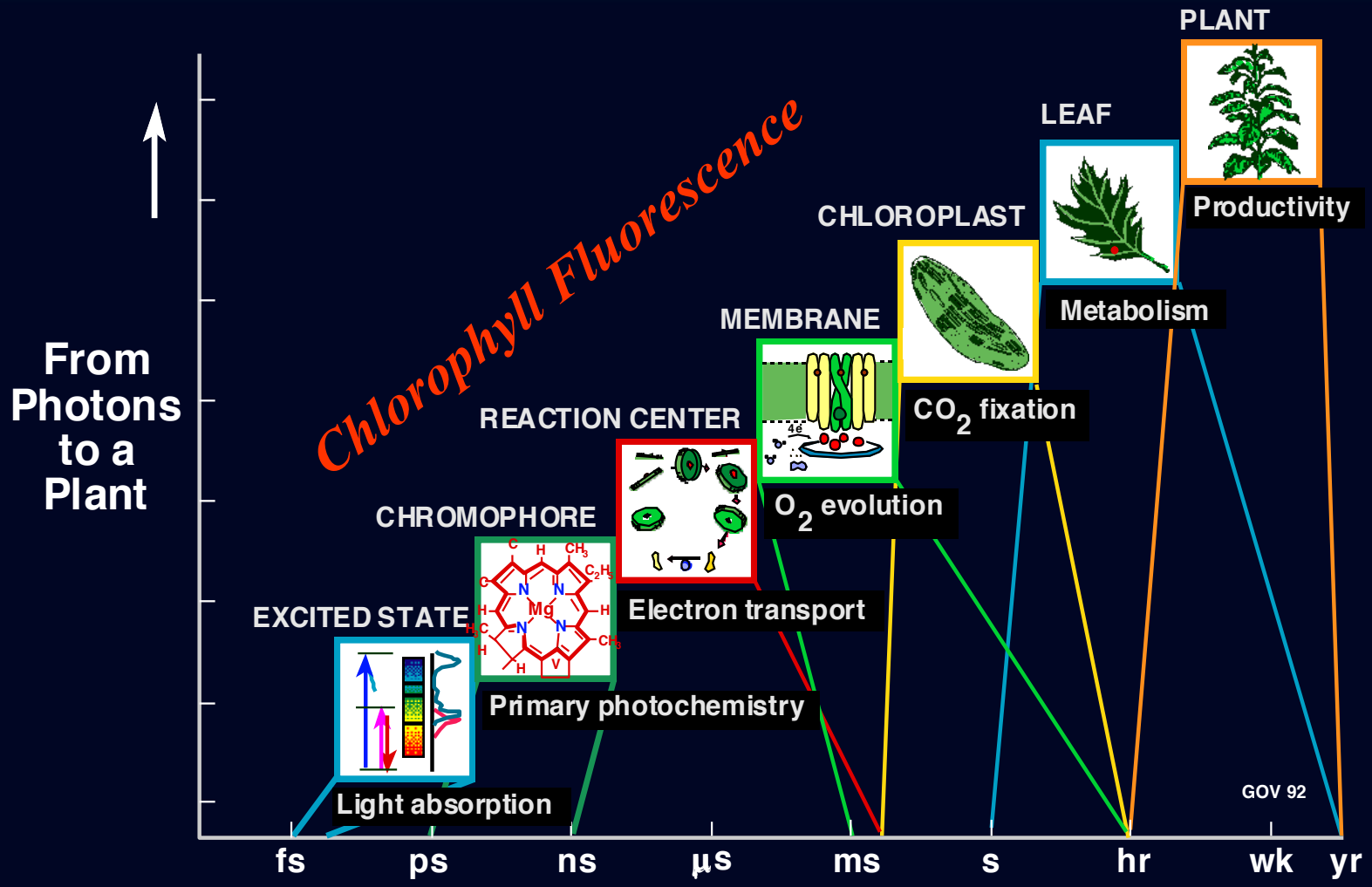
Chlorophyll Fluorescence

- Bioindicator, Biomarker, Biosensor, Physiological fingerprinting

- WHY ?

- Sensitive
- Reliable
- Non invasive
- Fast with reasonable cost
- Applicable in all living organisms with chlorophyll (plants, algae, mosses, lichens, animals etc.)

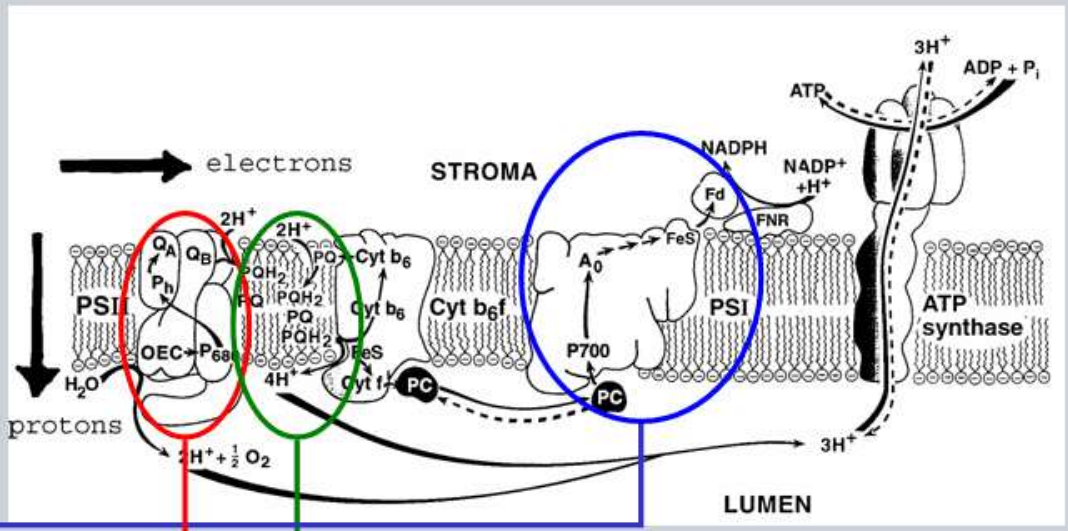
Time-scales : understanding plant response to stress

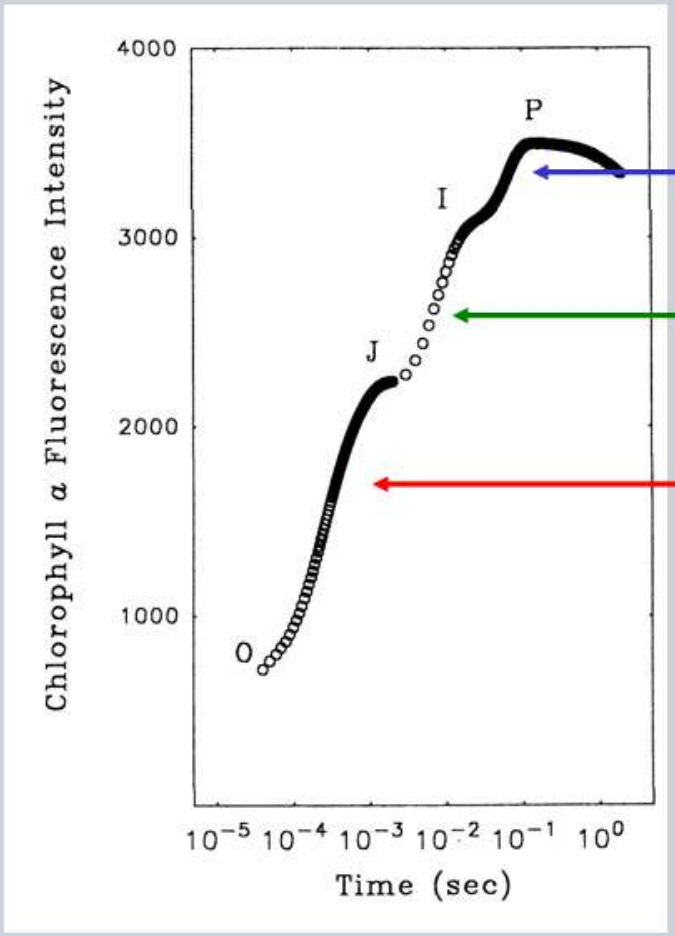


Modified after Osmond and Chow, Aust J Plant Physiol 1988

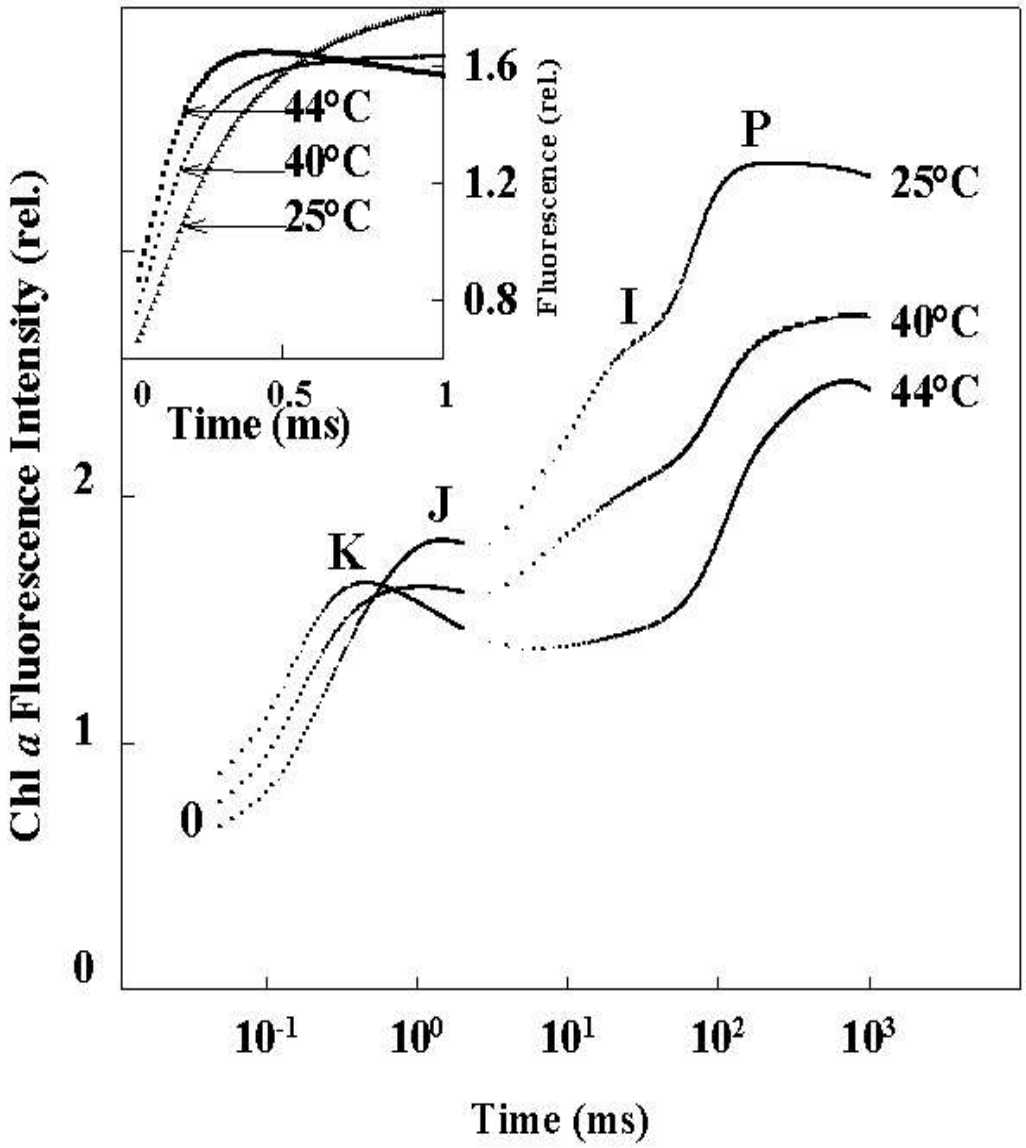
Prof. Reto Strasser-

Geneva University, Bioenergetics Lab.





A simplified interpretation of the relationship between OJIP-transient and electron acceptor pools of the electron transport chain.

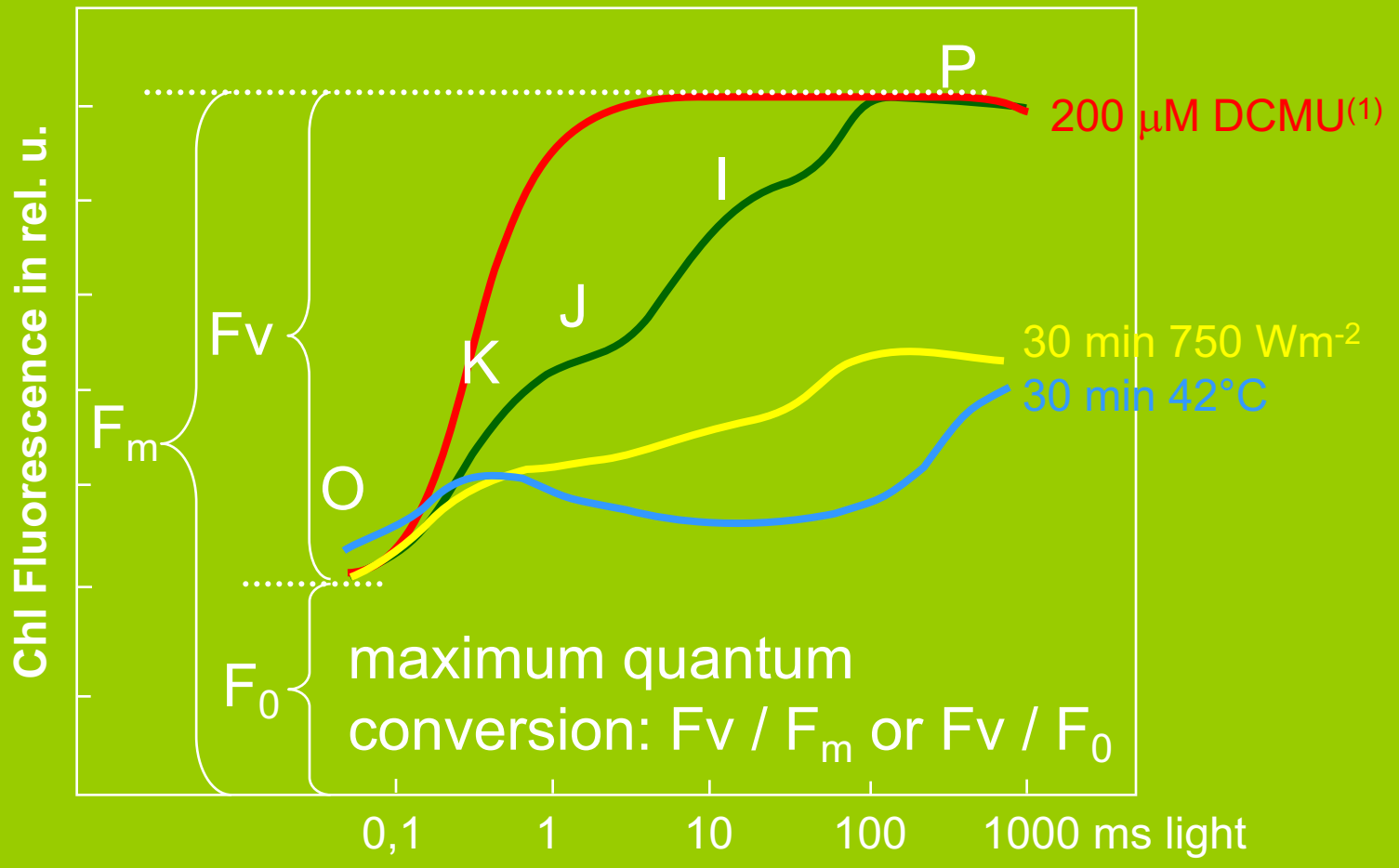


(O-J) phase corresponds to a complete reduction of the primary electron acceptor QA of PSII,

the release of fluorescence quenching during the (J-I) phase is controlled by the PSII donor side (water splitting activity).

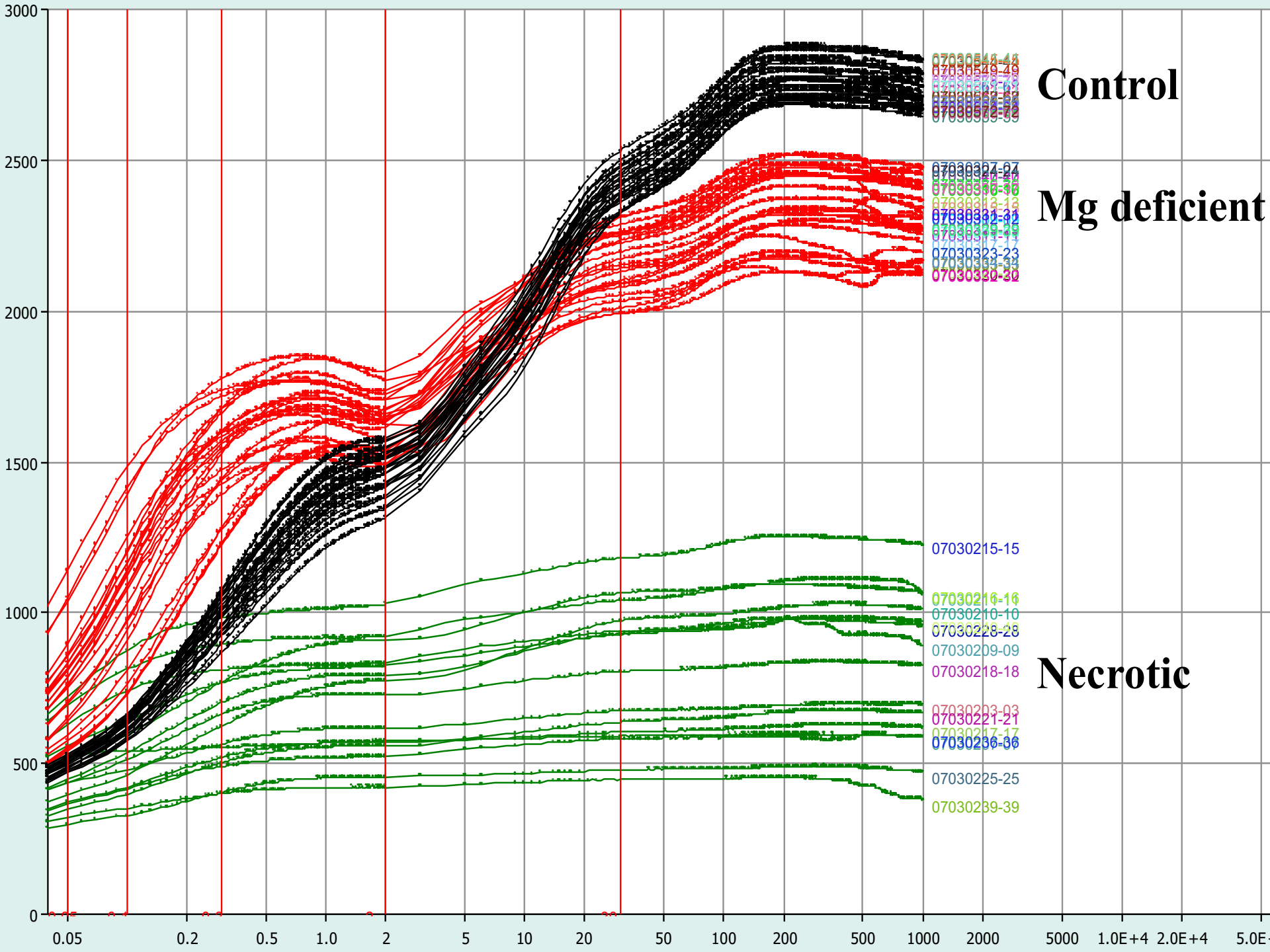
(I-P) corresponds to the release of fluorescence quenching by the oxidised plastoquinone pool

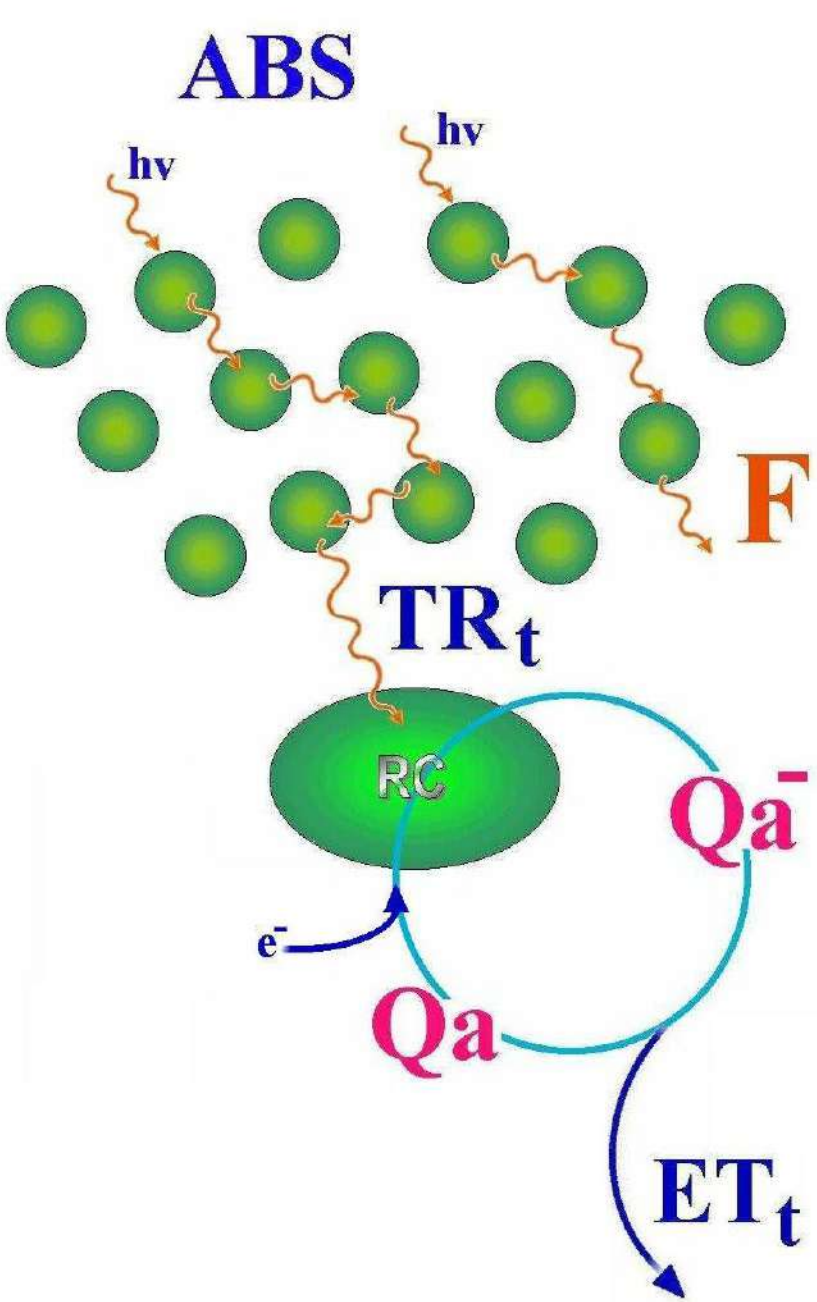
Fluorescence Rise



Srivastava A, Strasser RJ (1999) in: Crop Improvement for Food Security (Behl RK et al. eds.) SSARM, HISAR, pp 60-71

(1)Haldimann P, Strasser RJ (1999) Photosynthesis Research 62: 67-83


Control
Mg deficient
Necrotic
07030215-15
07030216-16
07030210-10
07030228-28
07030209-09
07030218-18
07030203-03
07030221-21
07030236-36
07030225-25
07030239-39
3000
2500
2000
1500
1000
500
0
0.05
0.2
0.5
1.0
2
5
10
20
50
100
200
500
1000
2000
5000
1.0E+4
2.0E+4
5.0E+



$$\frac{ABS}{RC}$$

$$\frac{TR_o}{RC}$$

$$\frac{ET_o}{RC}$$

$$\frac{TR_o}{ABS} = \varphi$$

$$\frac{ET_o}{TR} = \psi_o$$

$$\varphi_{Eo} \; \frac{ET_o}{ABS}$$

Derived JIP-test parameters table

Technical parameters

Slope at the origin of the fluorescence rise	M_O	$= (F_{300\mu s}-F_O) / (F_M-F_O)$
Relative variable fluorescence at 2 ms	V_J	$= (F_{2ms}/F_O) / (F_M-F_O)$

The specific fluxes (expressed per RC - reaction center)

Absorption, per RC	**ABS/RC**	$= (M_O/V_J) / ((1-F_O/F_M))$
Trapping at time zero, per RC	**TRo/RC**	$= M_O/V_J$
Dissipation at time zero, per RC	**DIo/RC**	= (ABS/RC) - (TRo/ABS)
Electron transport at time zero, per RC	**ETo/RC**	$= (M_O/V_J) (1-V_J)$

The phenomenological fluxes (expressed per CS – cross section of the leaf tissue)

Absorption, per CS	**ABS/CS**	$= (TR_O/ABS) / (ABS/CS)$
Trapping at time zero, per CS	**TRo/CS**	= (TRo/ABS) (ABS/CS)
Dissipation at time zero, per CS	**DIo/CS**	= (ABS/CS) - (TRo/CS)
Electron transport at time zero, per CS	**ETo/CS**	$= (M_O/V_J) (1-V_J)$

The yields (or fluxes ratios)

Maximum quantum yield of primary photochemistry	φ_{Po}	$=$ TRo/ABS $= (F_M-F_O) / F_M$
Probability that a traped exciton moves an electron further than Q_A^-	Ψ_o	$=$ ETo/TRo $= 1 - V_J$
Probability that an absorbed photon moves an electron further than Q_A^-	$\varphi_{Eo}= \varphi_{Po}\,\Psi_o$	$=$ (TRo/ABS) (ETo/TRo) $=$ ETo/ABS $= (1-F_O/F_M) (1-V_J)$

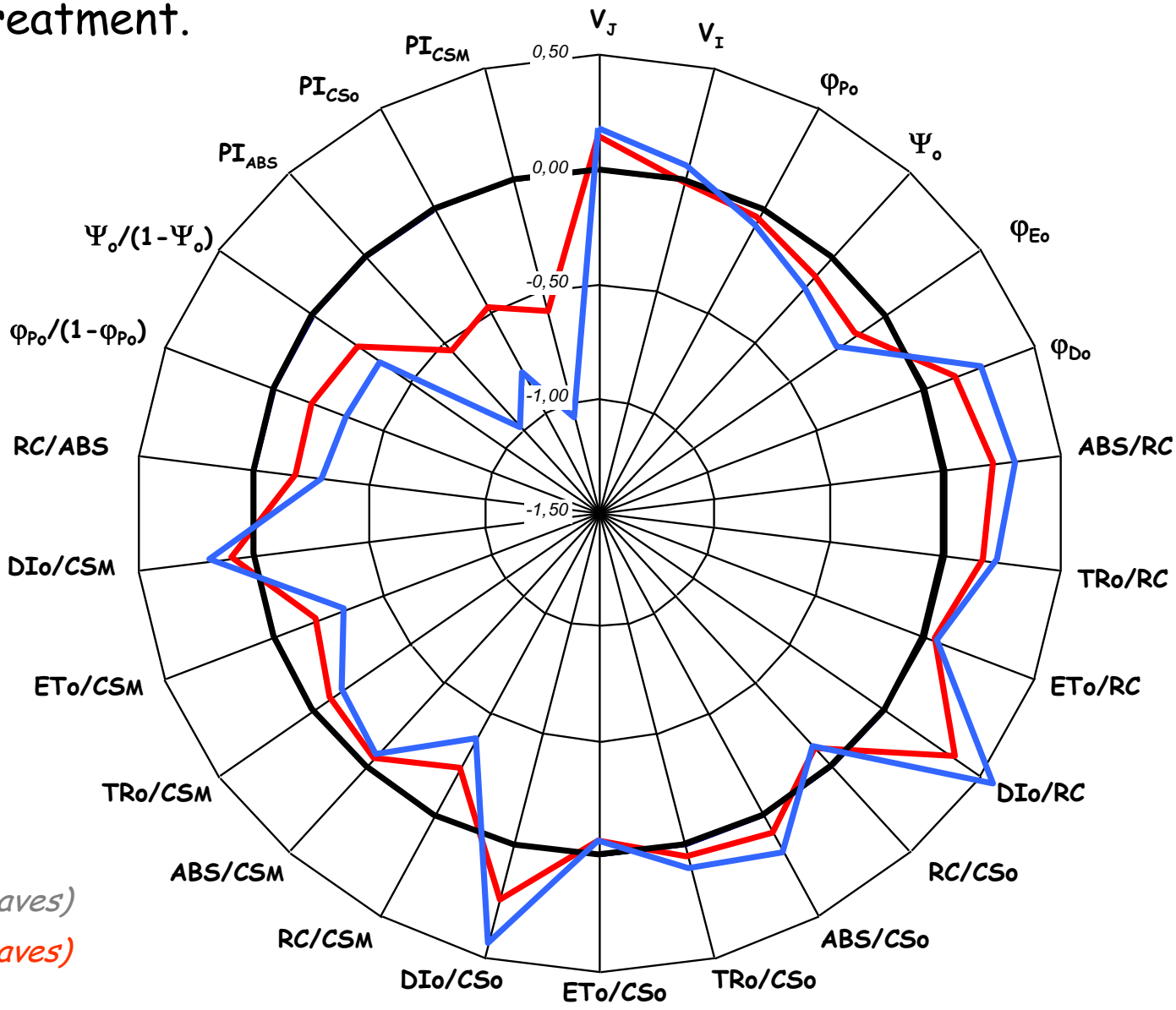
Vitality Indexes

Density RCs per chlorophyll	**RC/ABS**
Conformation term for primary photochemistry	$(\varphi_{Po}/(1 - \varphi_{Po}))$ $=$ TRo/DIo $= F_O/F_M$
Conformation term for the thermal reactions (non light depending reactions)	$(\Psi_o /(1 - \Psi_o))$ $=$ ETo/(dQ_A^-/dt_0)
Performance Index	$PI_{ABS} = [RC/ABS] [\varphi_{Po} /(1 - \varphi_{Po})] [\Psi_o /(1 - \Psi_o)]$
Driving force on a chlorophyll basis	ΔF_{ABS} $=$ log $[PI_{ABS}]$

These graphics present the constellation of selected JIP-test parameters which quantify the behaviour of plants exposed to different stress treatment.

Siper-plot representation. Variations of the normalised JIP-test parameters by the respective control. More precisely, the nutritional stress linked to a lack of B and Mg is regarded as a deviation of the reference state and considered as non stress (for which the control values turn on a circle with a radius of 100%).

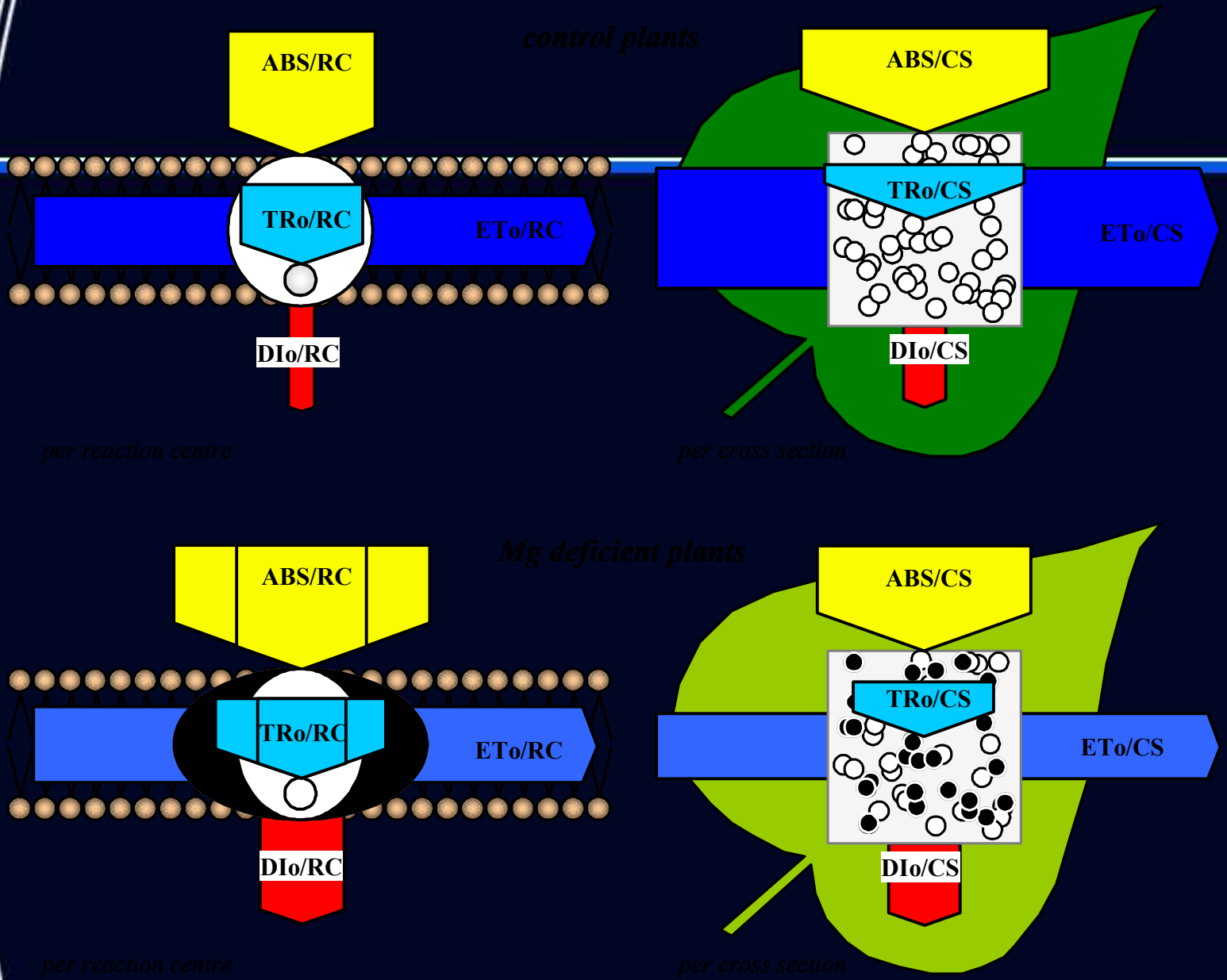
Boron deficiency first appears on the youngest leaves whereas magnesium deficiency can be detected on the oldest leaves.



Respective control

B deficiency (youngest leaves)

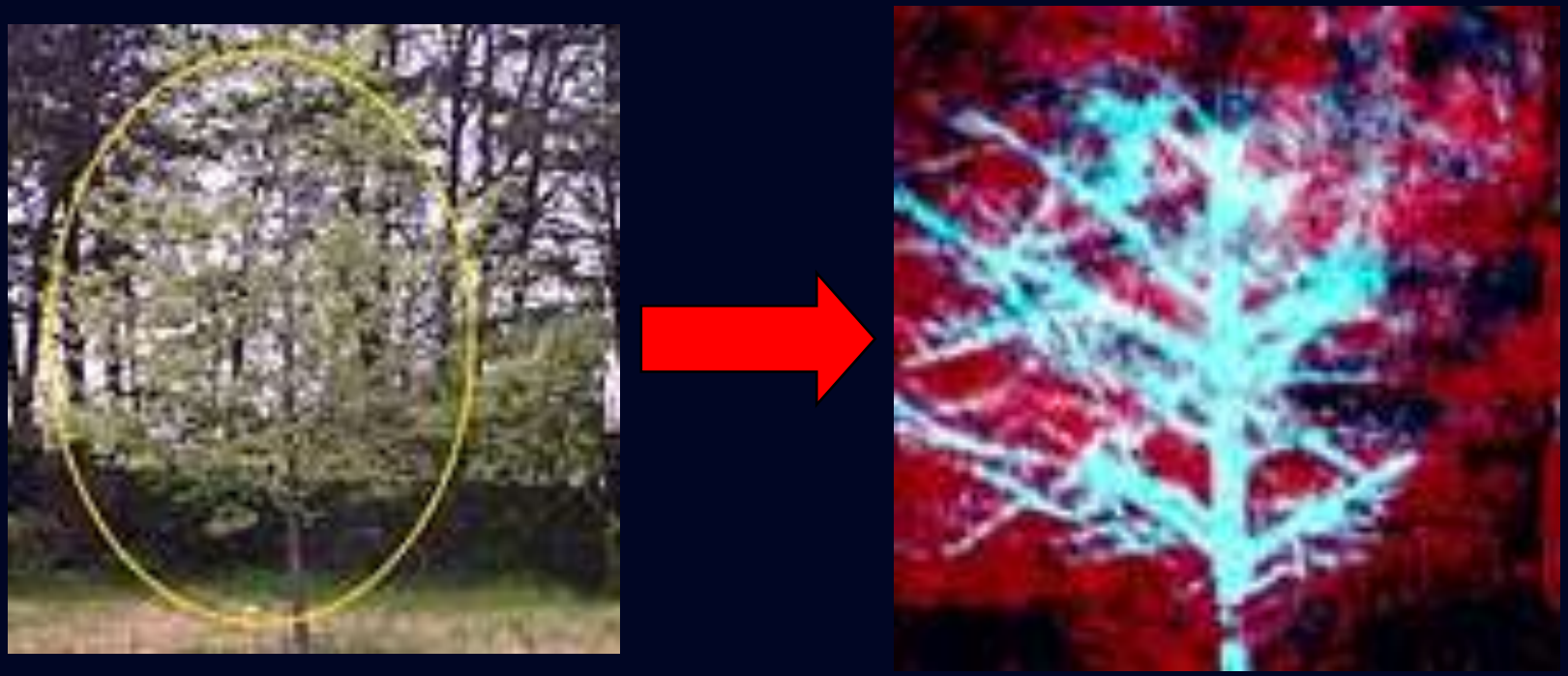
Mg deficiency (mature leaves)


control plants
ABS/RC
TRo/RC
ETo/RC
DIo/RC
per reaction centre
ABS/CS
TRo/CS
ETo/CS
DIo/CS
per cross section
Mg deficient plants
ABS/RC
TRo/RC
ETo/RC
DIo/RC
per reaction centre
ABS/CS
TRo/CS
ETo/CS
DIo/CS
per cross section

Some examples of recent applications

Plant stress prediction - University of Geneva, Switzerland

chlorophyll fluorescence in terrestrial vegetation- trees physiological state



European Space Agency (http://www.esa.int/esaLP/SEM2VSBE8YE_index_0.html)

Fruit quality estimation

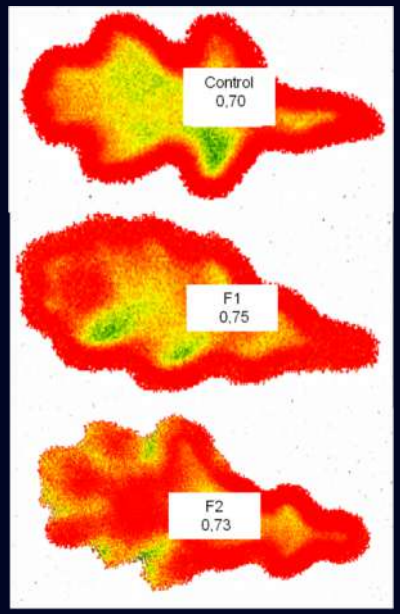
Fruit quality estimation

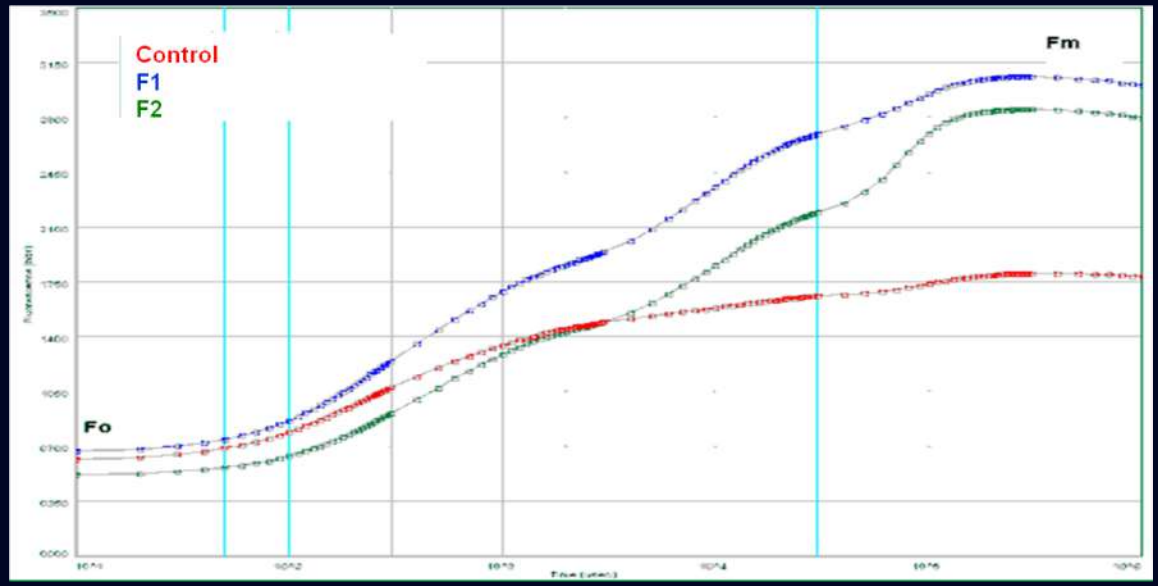
Fruit quality estimation

Water Quality _Oak

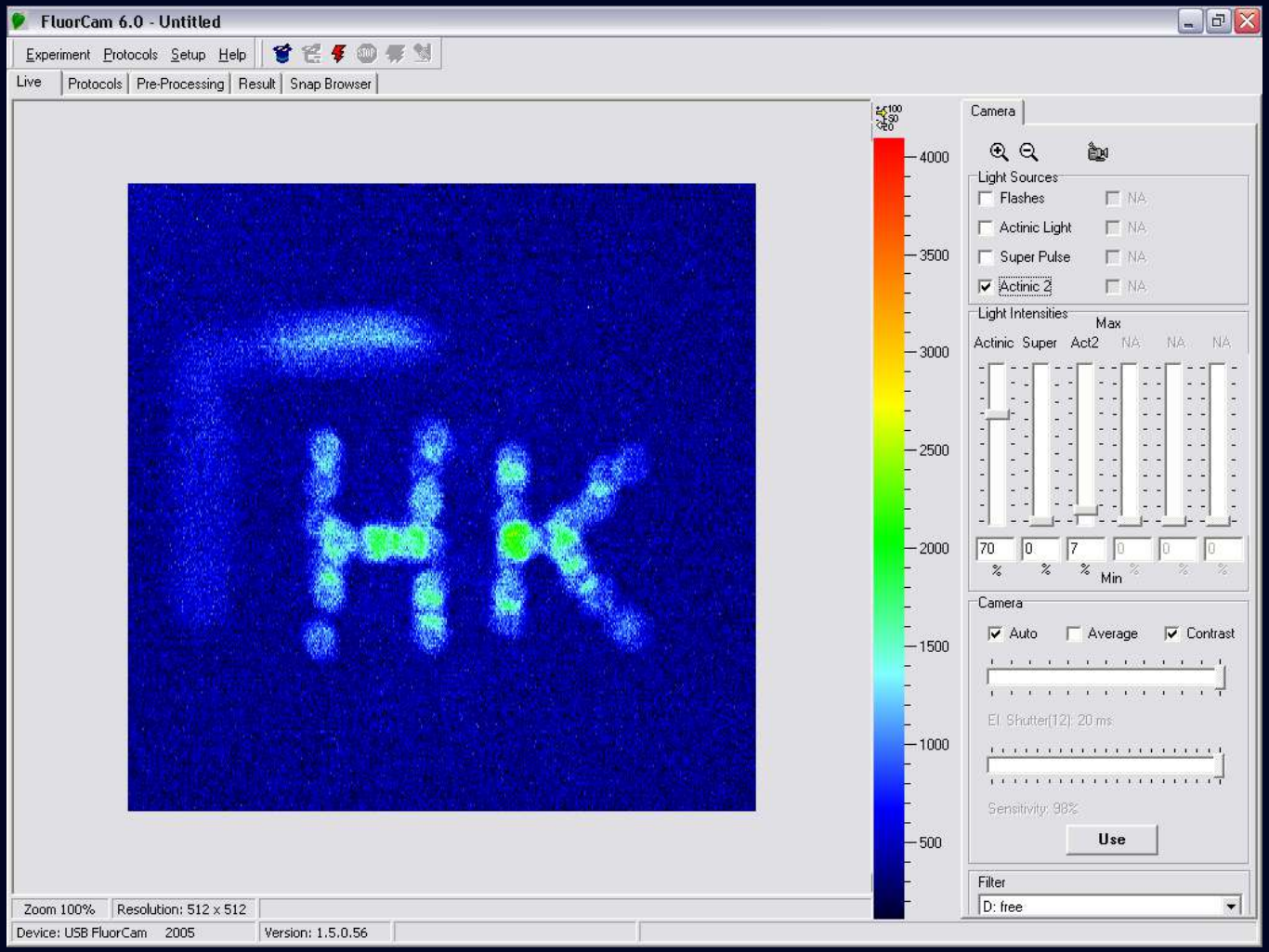




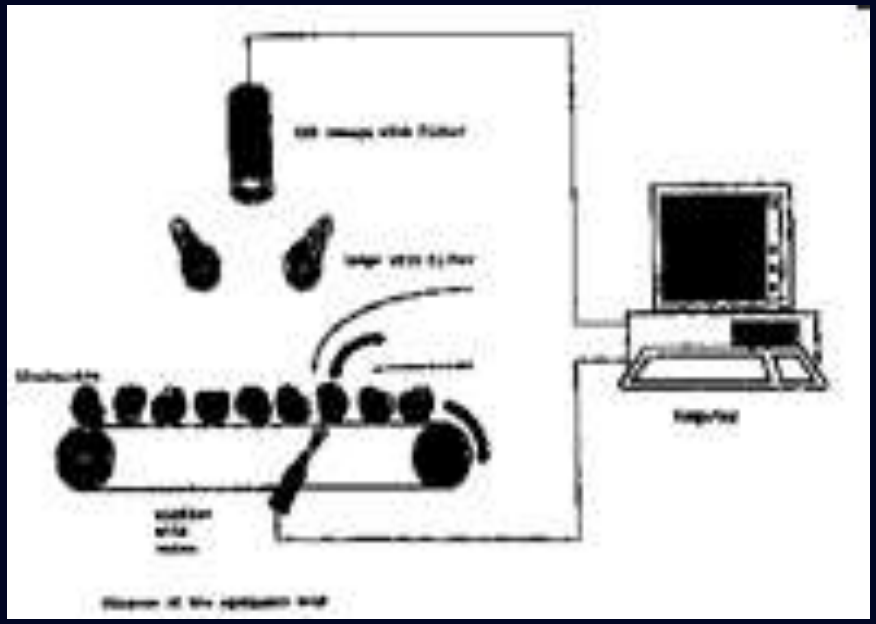


Seed quality



SORTING

Jalink, Hendrik (NL); Schoor, Rob van der (NL); Bino, Raoul John (NL).

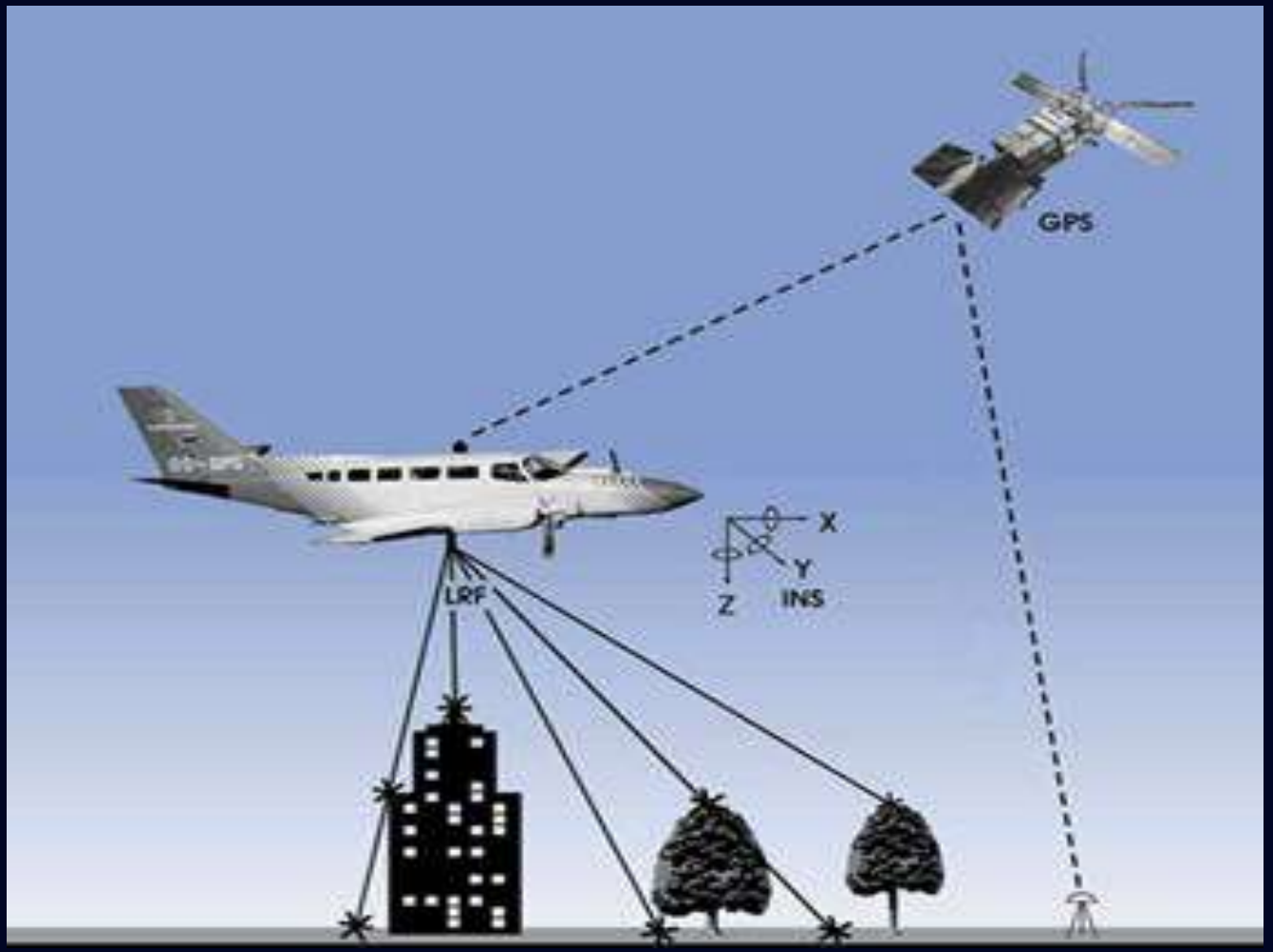


Wageningen University and Research Centre

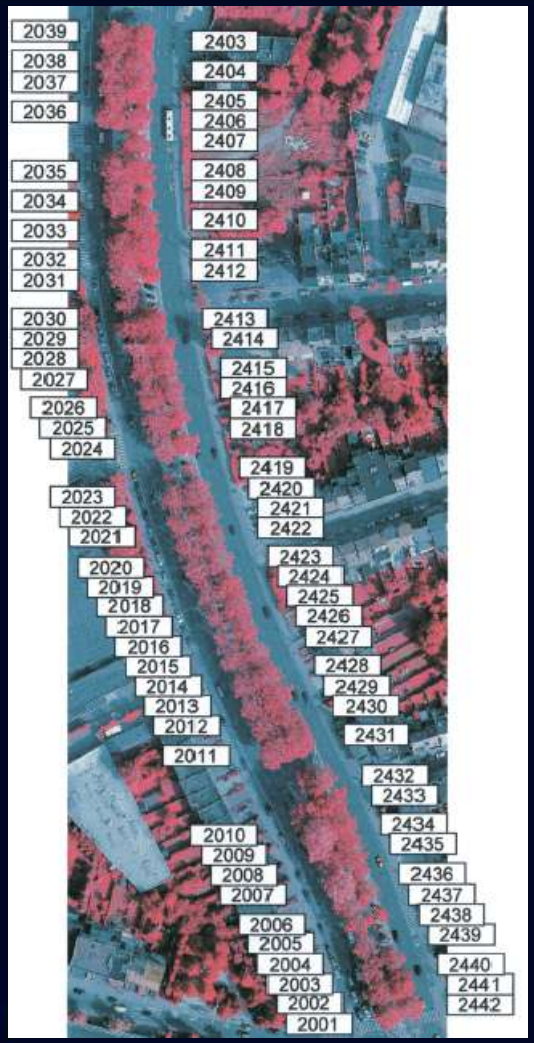
SPORTS FACILITIES (Euro 2012, Poland / Ukraine)

India- *Himalaya*

Chlorophyll Fluorescence

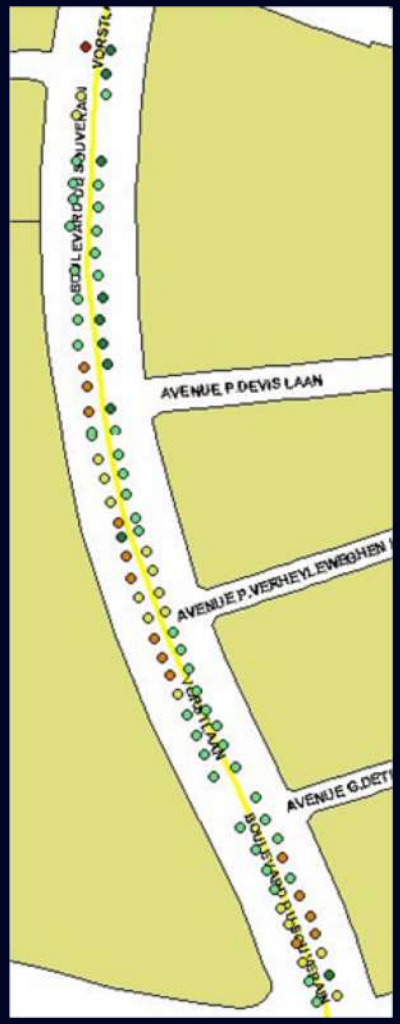


Airborne Remote Sensing



Quality assessment of urban trees: A comparative study of physiological characterisation, airborne imaging and on site fluorescence monitoring by the OJIP-test

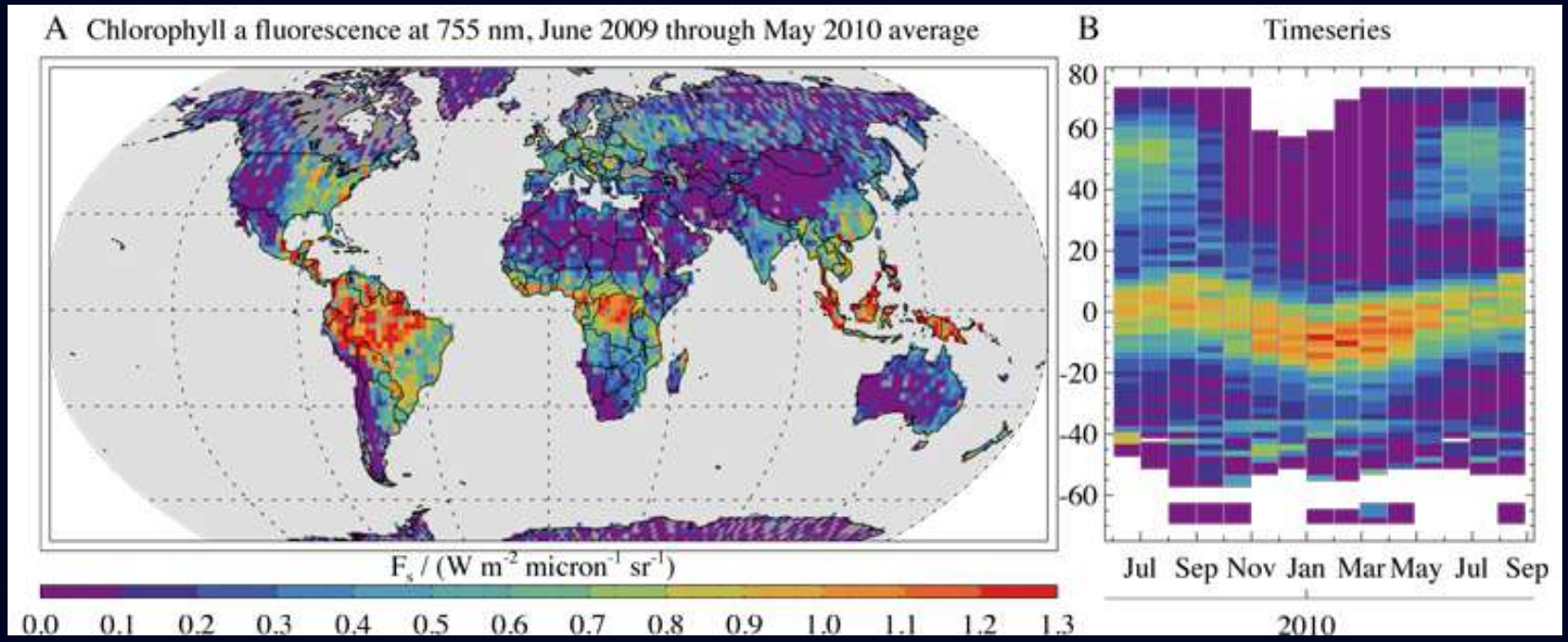
Hermans C et al. Journal of Plant Physiology Volume 160, Issue 1, 2003, Pages 81-90



Leopold Park, Brussels - Belgium

Precision farming and agriculture

First-of-its-kind fluorescence map offers a new view of the world's land plants



Scientists from NASA's Goddard Space Flight Center in Greenbelt, Md. (physorg.com)

SPACE STATIONS

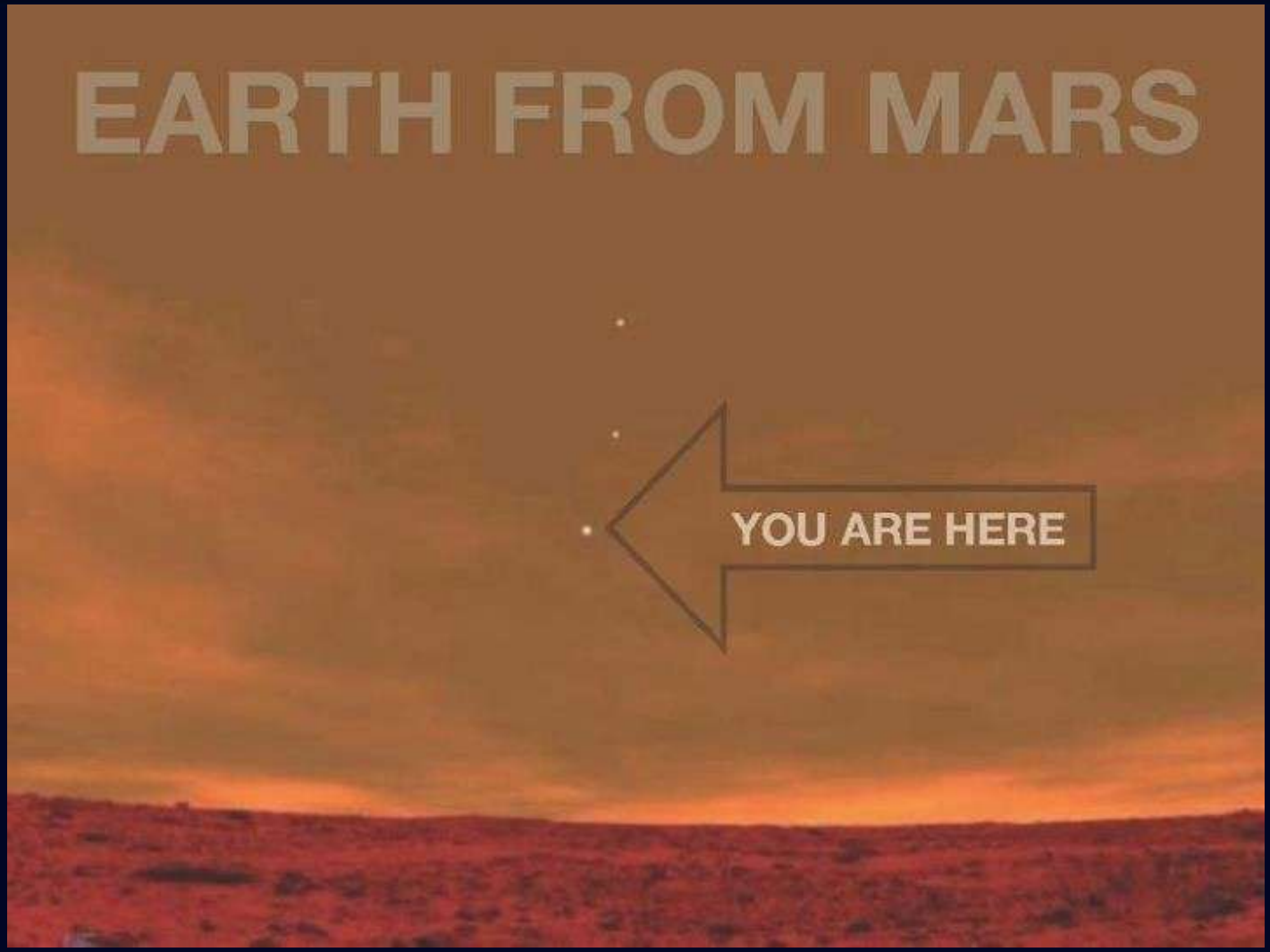
NASA- Space Life Sciences Laboratory, Kennedy Space Center, Florida, USA

Probing the Responses of Plants by Chlorophyll Fluorescence under Controlled Environments

System Produkcji Biomasy
w przestrzeni kosmicznej

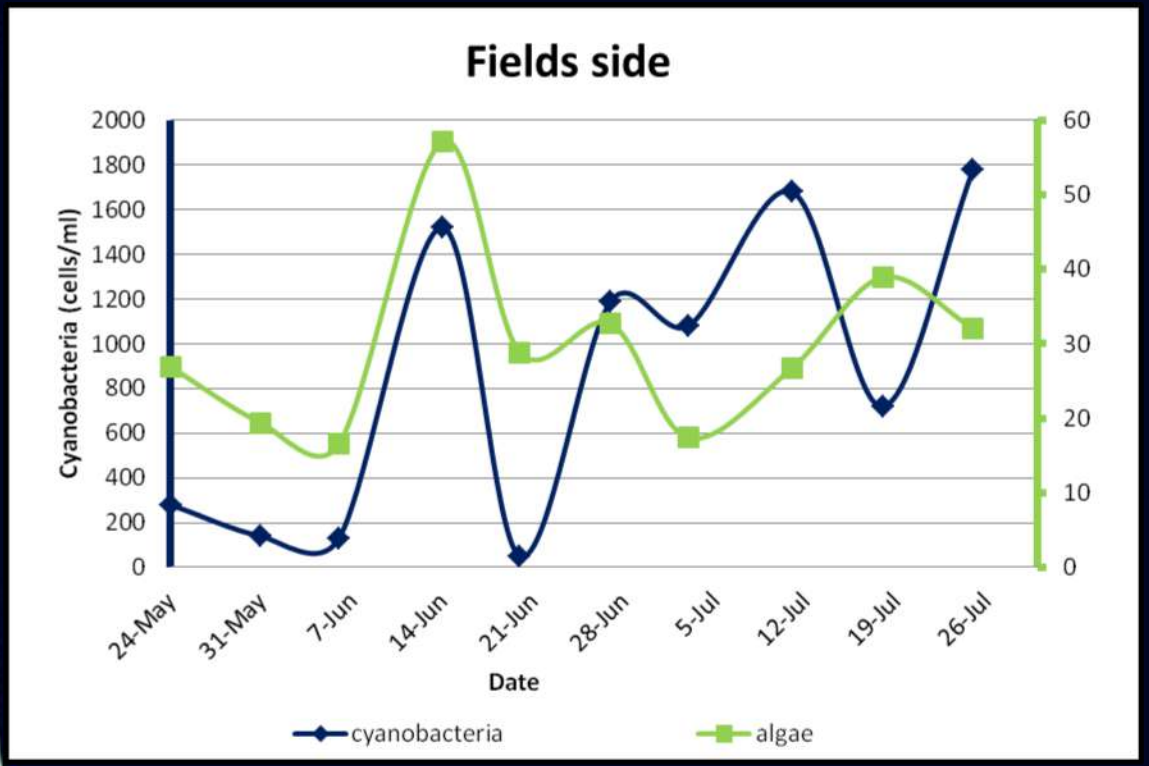
Wnętrze komory wzrostu do
produkcji biomasy

Discovering life on Mars

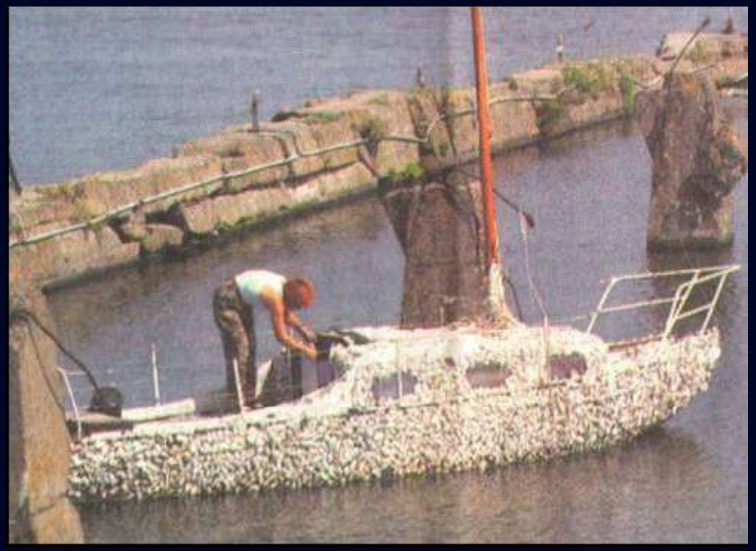


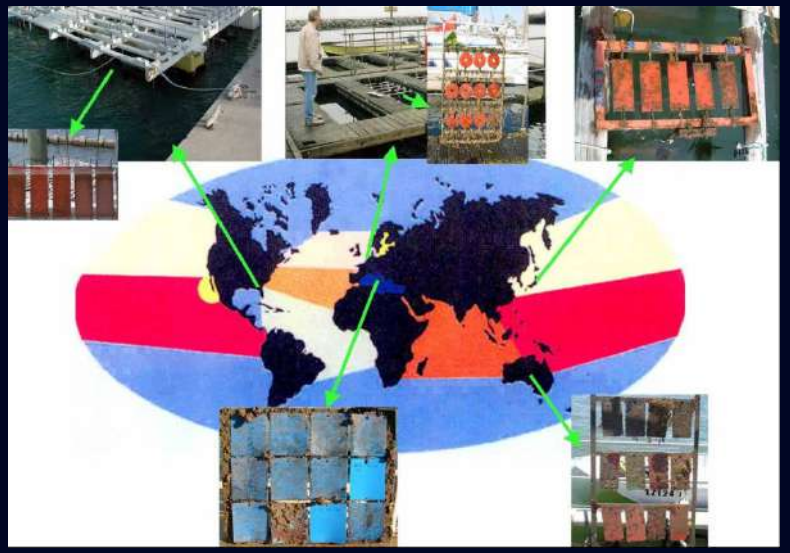
Water Ecosystems Applications

Water Quality: lake, rivers and oceans



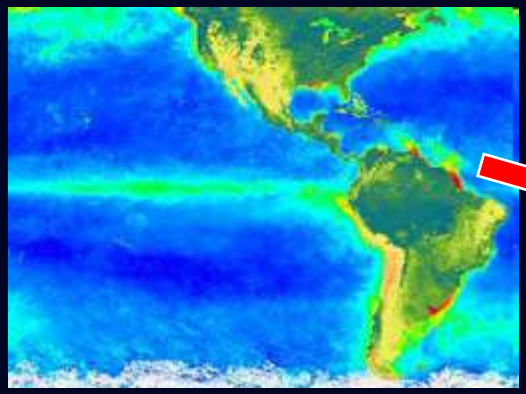
Biofilm on boats and ships

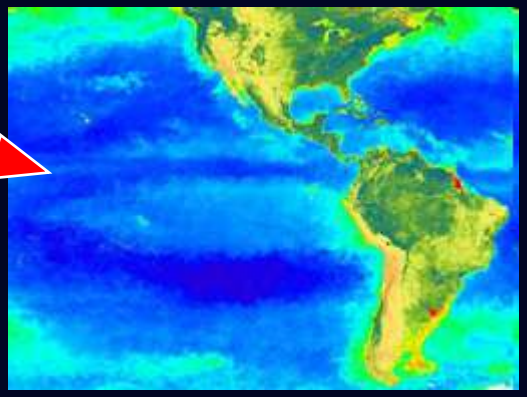


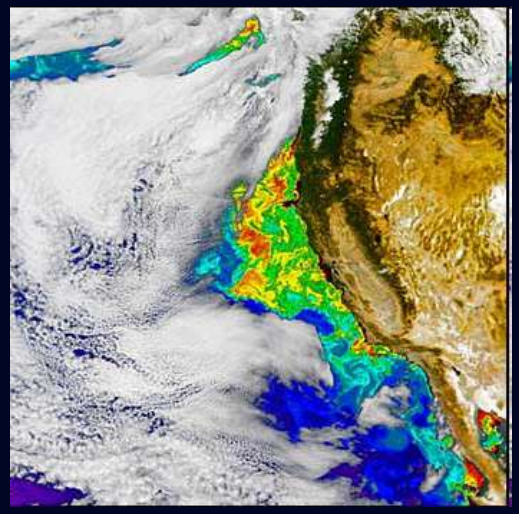


Jebel Ali Power and Desalination Station

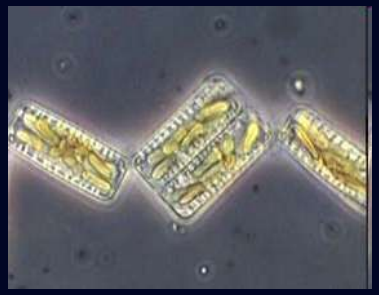
National Alarm System (Modis – NASA)

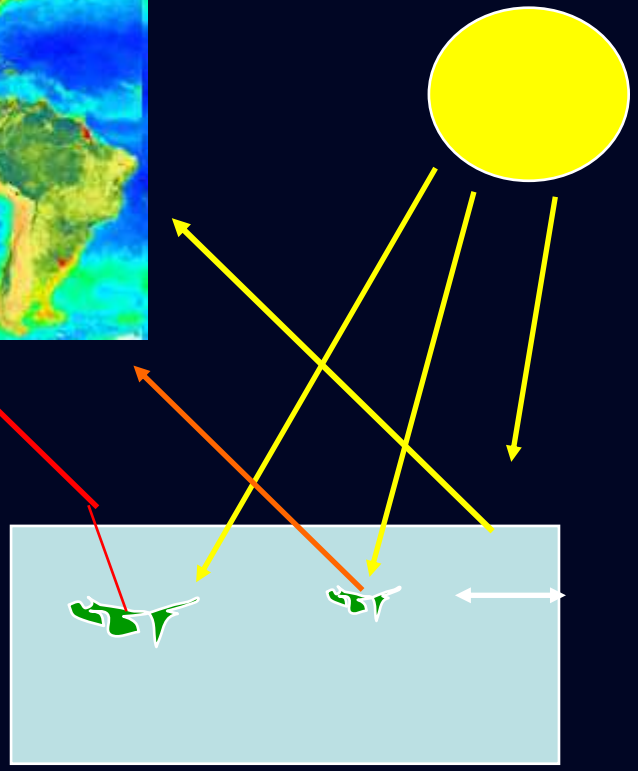






Algae



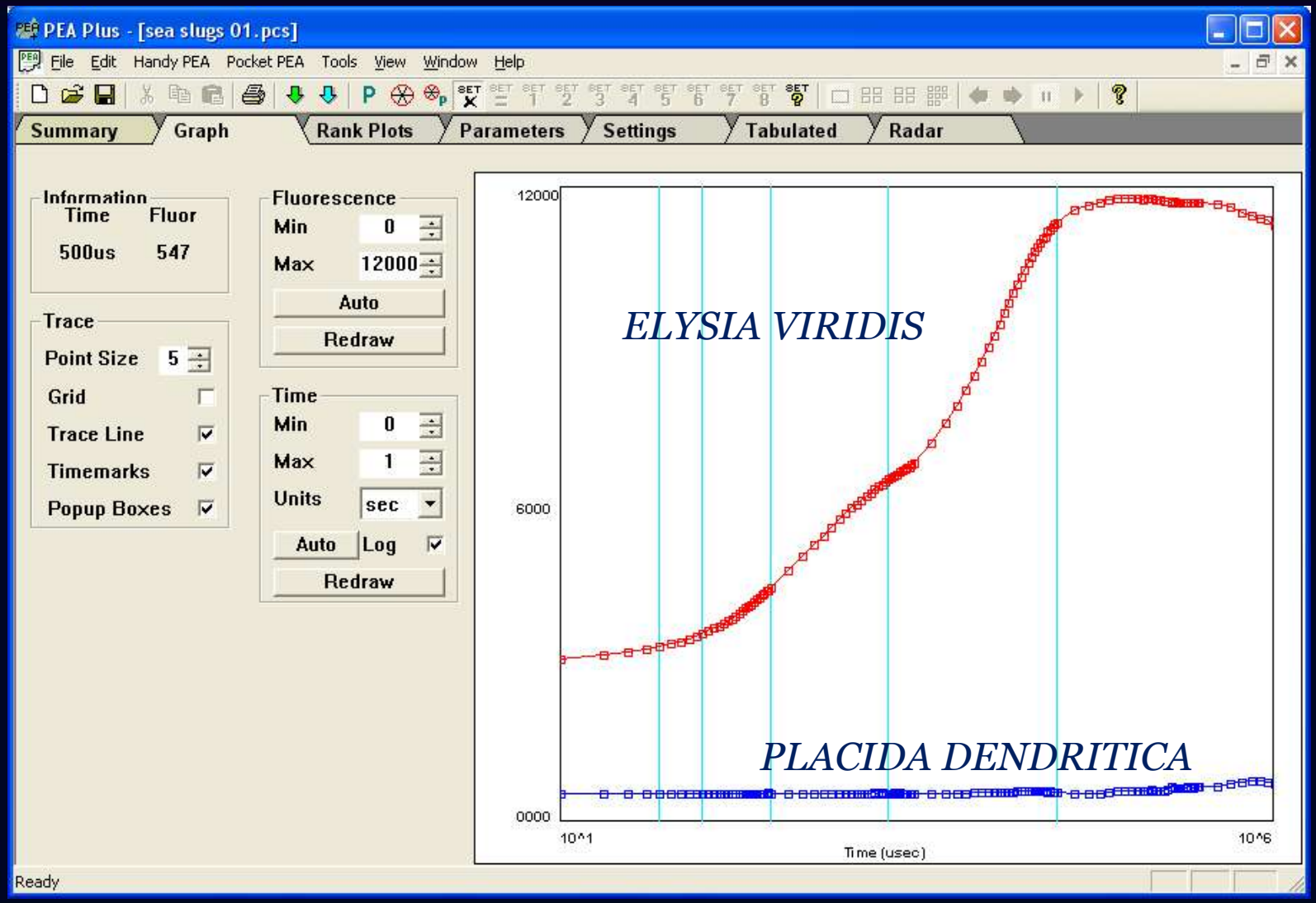


http://daac.gsfc.nasa.gov/oceancolor/scifocus/oceanColor/warming.shtml
http://oceancolor.gsfc.nasa.gov/

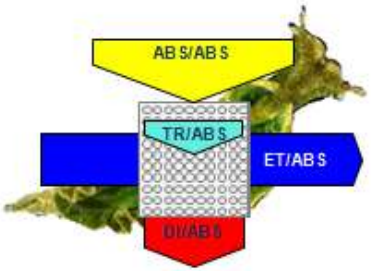
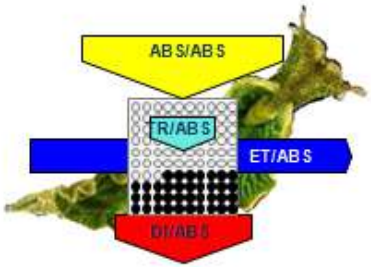
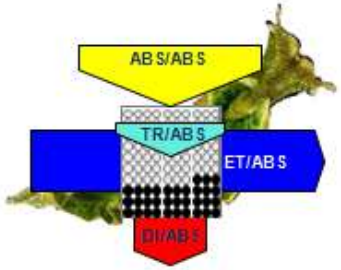
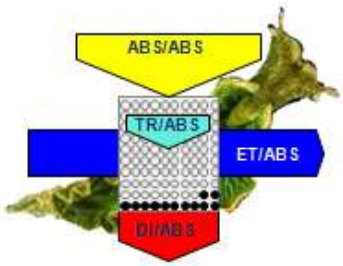
Sea slug vitality assessment
De Aveiro University, Portugal

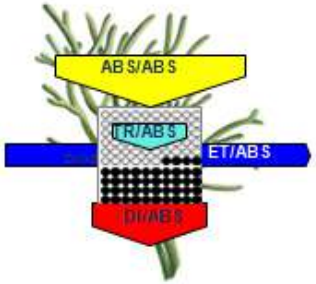
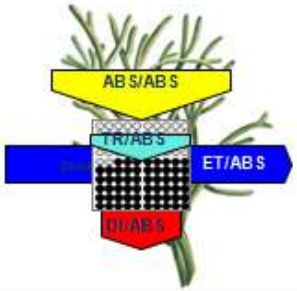
universidade de aveiro

Dr. Sonia Cruz

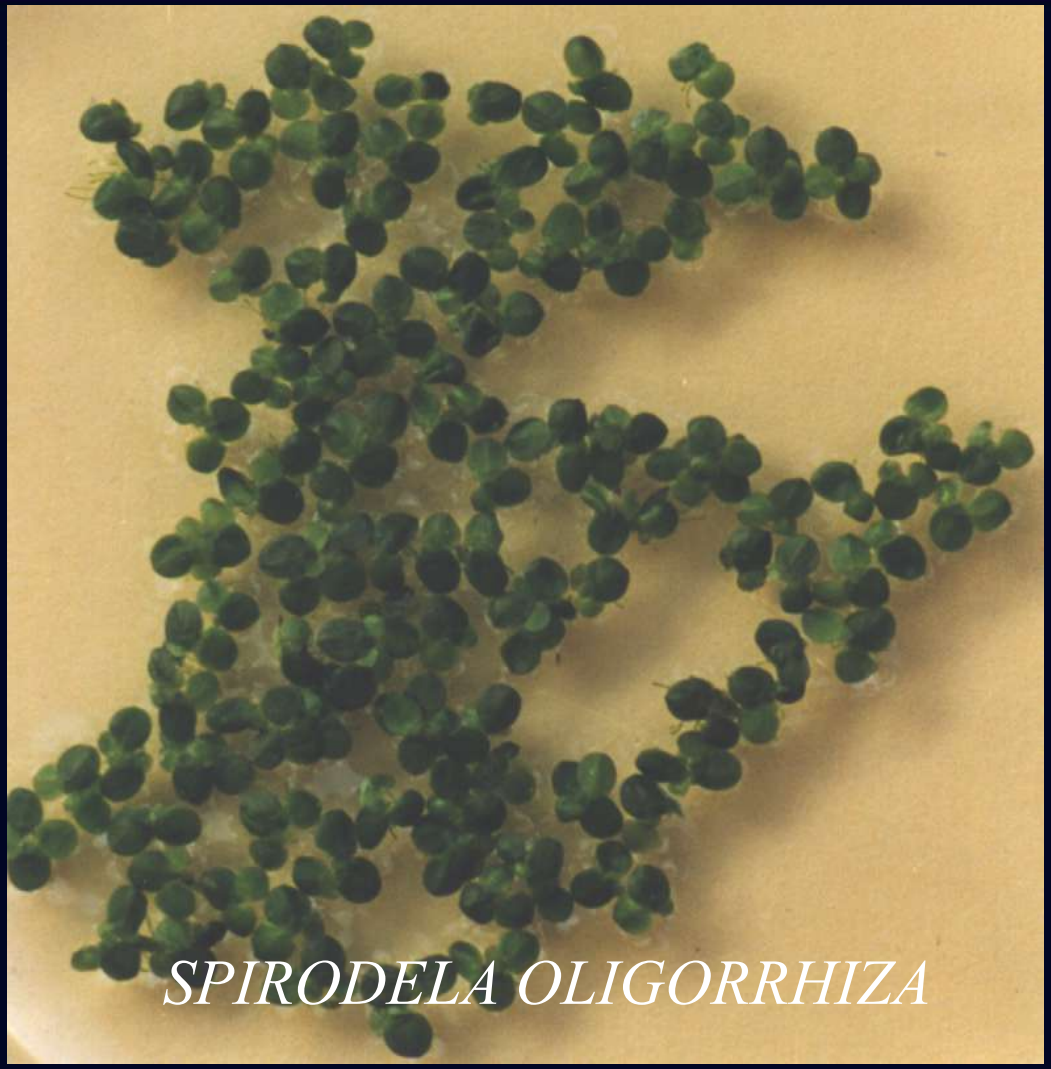

PEA Plus - [sea slugs 01.pcs]
File Edit Handy PEA Pocket PEA Tools View Window Help
Summary Graph Rank Plots Parameters Settings Tabulated Radar
Information
Time Fluor
500us 547
Trace
Point Size 5
Grid
Trace Line
Timemarks
Popup Boxes
Fluorescence
Min 0
Max 12000
Auto
Redraw
Time
Min 0
Max 1
Units sec
Auto Log
Redraw
12000
6000
0000
10^1
10^6
Time (usec)
ELYSIA VIRIDIS
PLACIDA DENDRITICA
Ready

High and low light, with and without Codium

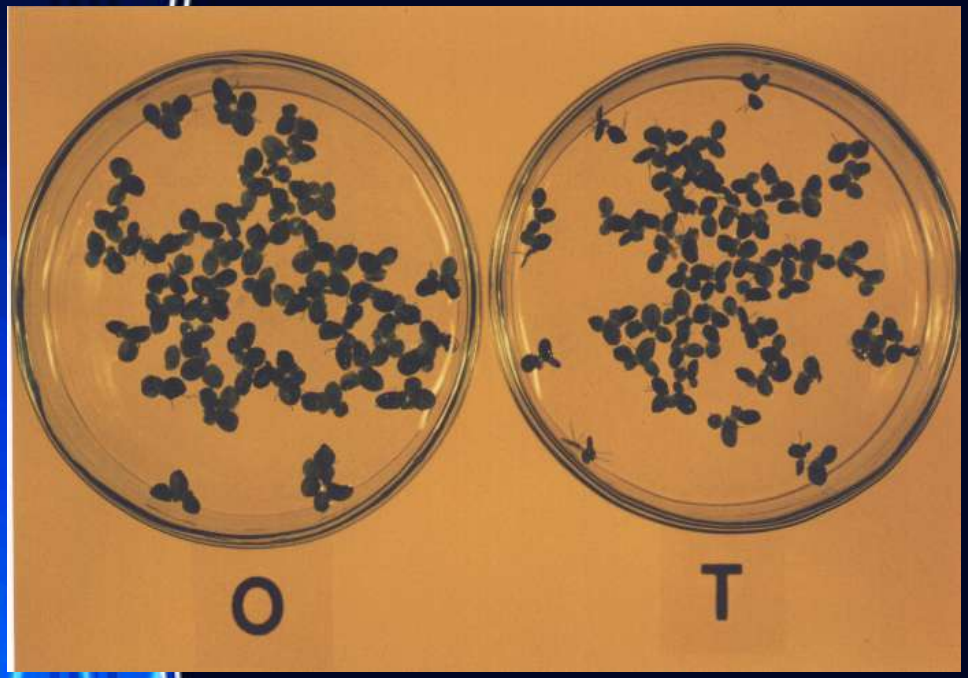
High Light		Low light	
With Codium	Without Codium	With Codium	Without Codium
			

Codium	
High light	Low light
	

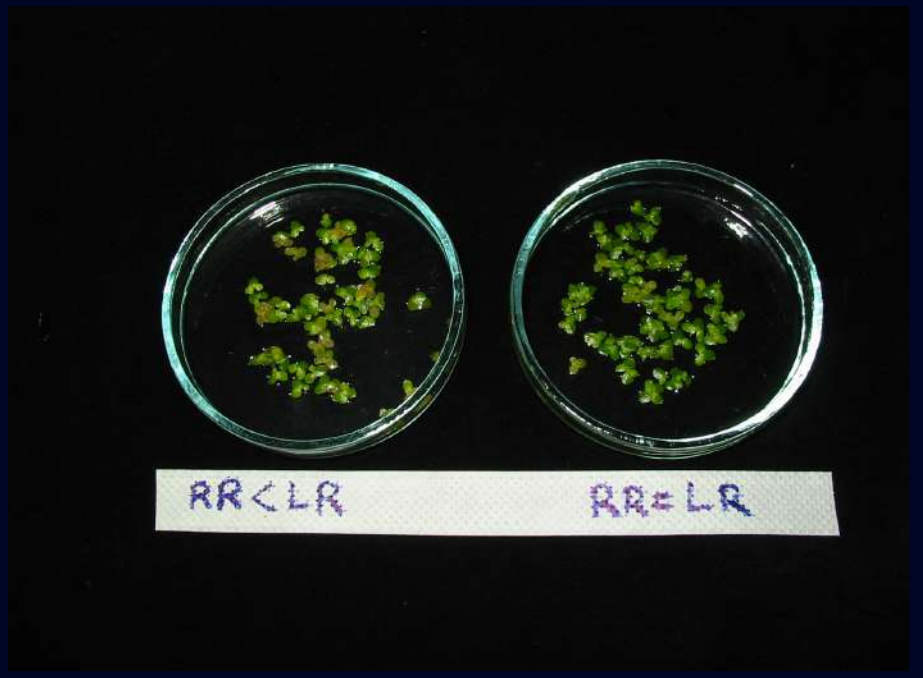
Kalaji H.M., Sytar O., Brestic M., Samborska I.A., Cetner M.D., Carpentier C. (2016) Risk Assessment of Urban Lake Water Quality Based on in-situ Cyanobacterial and Total Chlorophyll-a Monitoring. Pol. J. Environ. Stud. Vol. 25, No. 2 (2016), 1-7. DOI: 10.15244/pages/60895



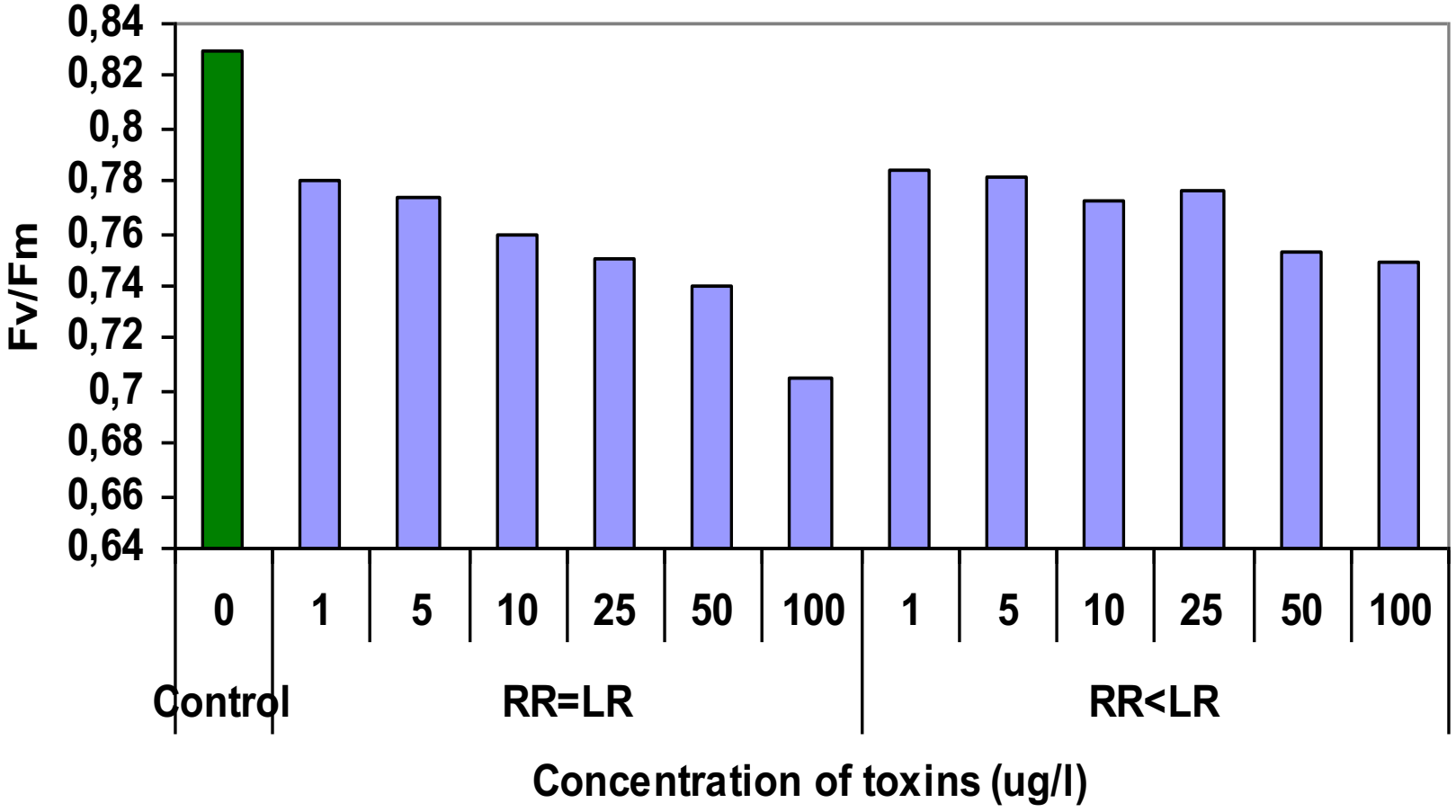
Toxins

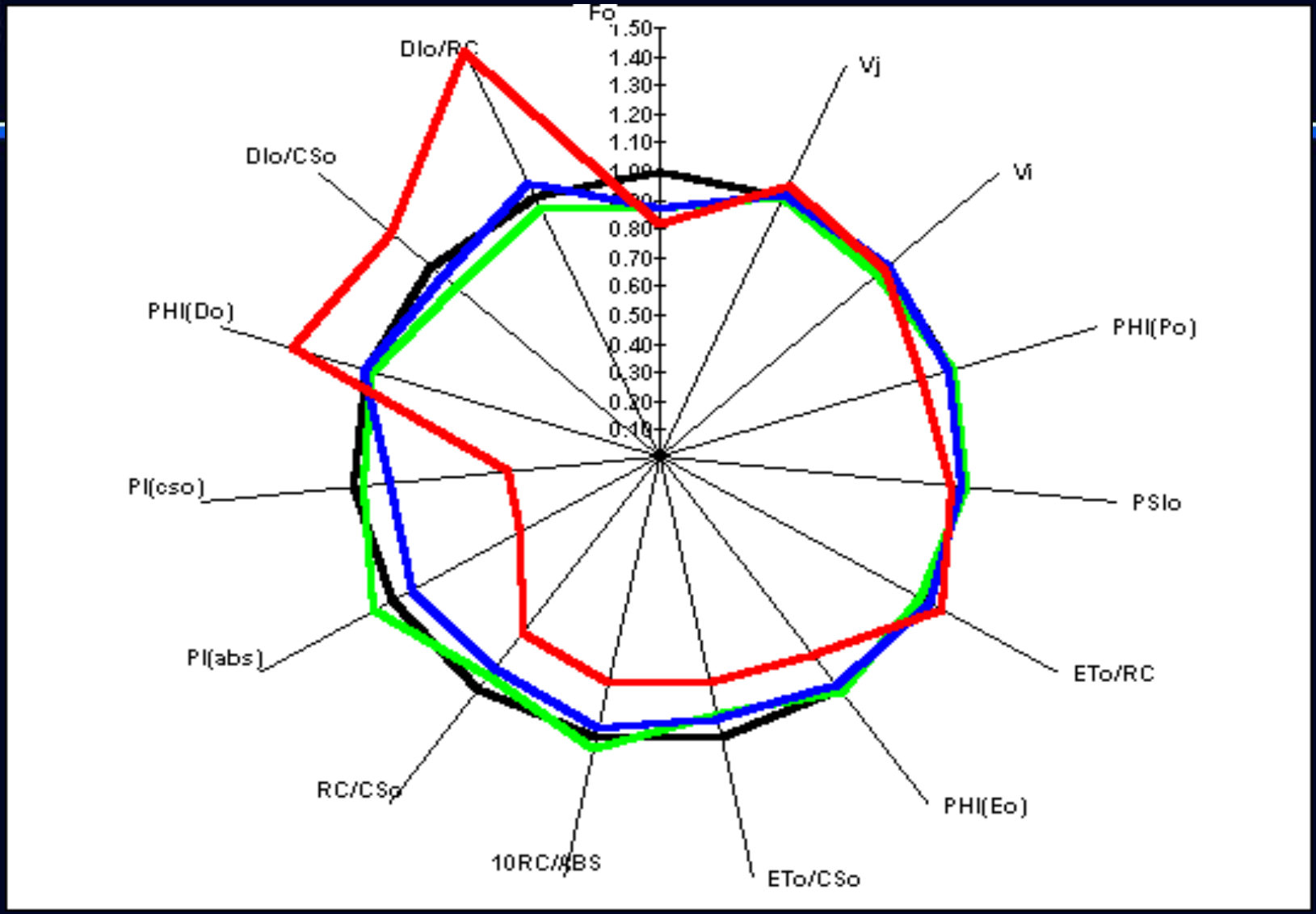


Spirodela oligorrhiza without (o) and with toxin MC-LR (T)



Spirodela oligorrhiza with toxins: MC-RR and MC-LR


0,84
0,82
0,8
0,78
0,76
0,74
0,72
0,7
0,68
0,66
0,64
Fv/Fm
0
1
5
10
25
50
100
1
5
10
25
50
100
Control
RR=LR
RR<LR
Concentration of toxins (ug/l)



Control 5µg L^{-1} 25 µg L^{-1} 100 µg L^{-1}

- **JIP-Test technique as biosensor for early detection of heavy metals effects on water plants (*Spirodela oligorrhiza*)**

Hazem M. Kalaji, Z. Romanowska-Duda, Reto J. Strasser

BIOLOGICAL LETT. 2005, 42(2): 191

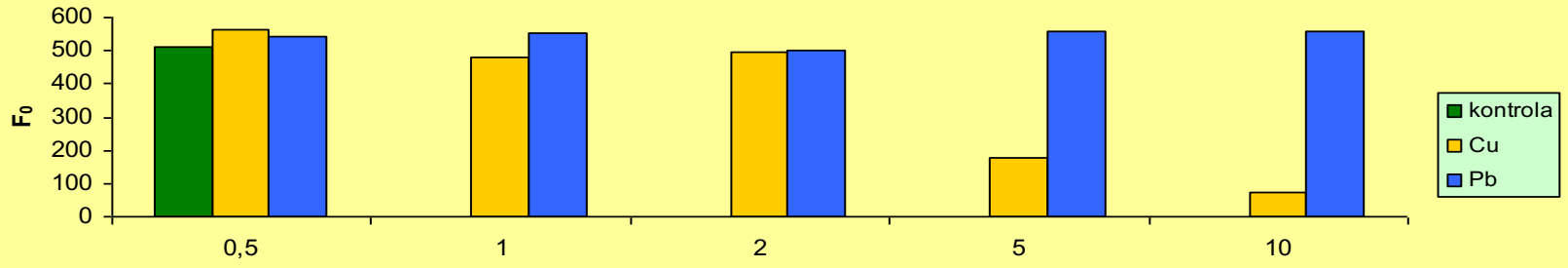
Spirodela oligorrhiza plants were grown under optimal conditions on a growth medium with or without the addition of heavy metals (Cu,Pb) ions during 24 hours to growth medium in the range from 0 to 10 ppm

Heavy metals

Spirodela oligorrhiza with different concentrations of Cd

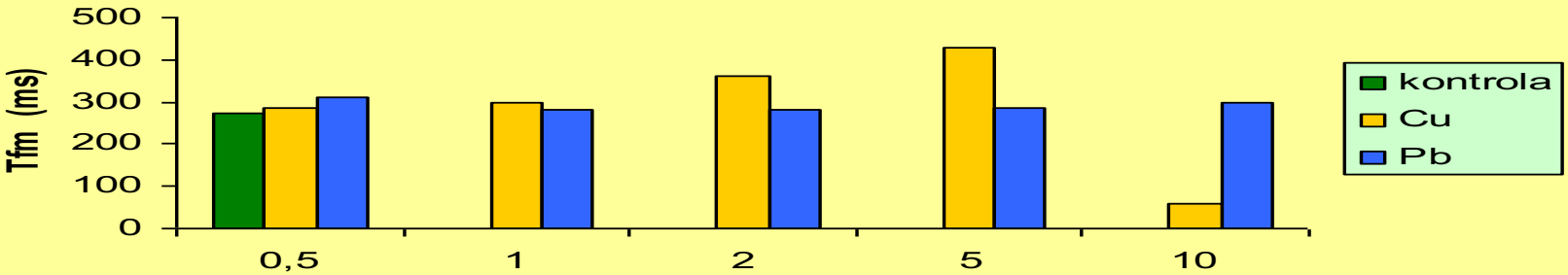
Spirodela oligorrhiza with different concentrations of Cu

Minimal Fluorescence (Fo)



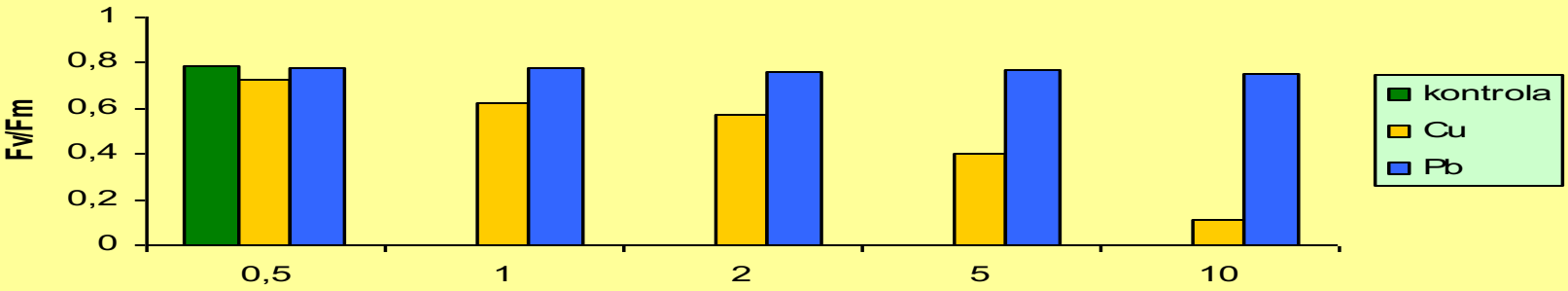
Heavy metals concentration (ppm)

Time to reach maximal fluorescence (Tfm)

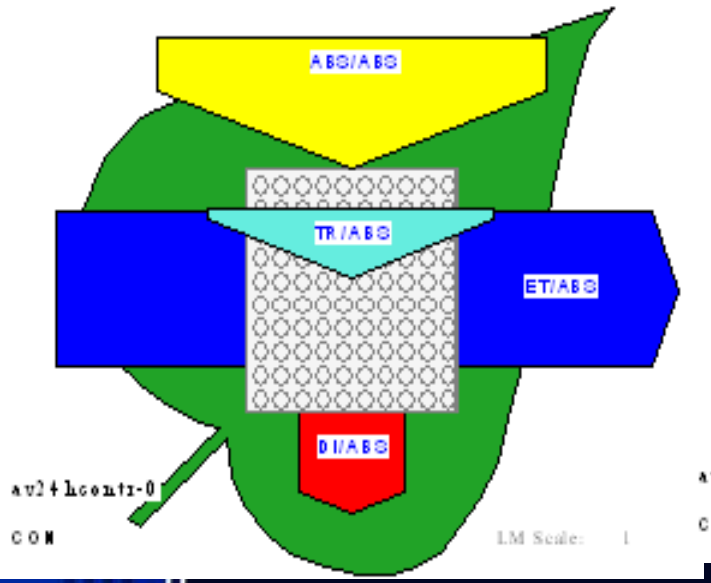


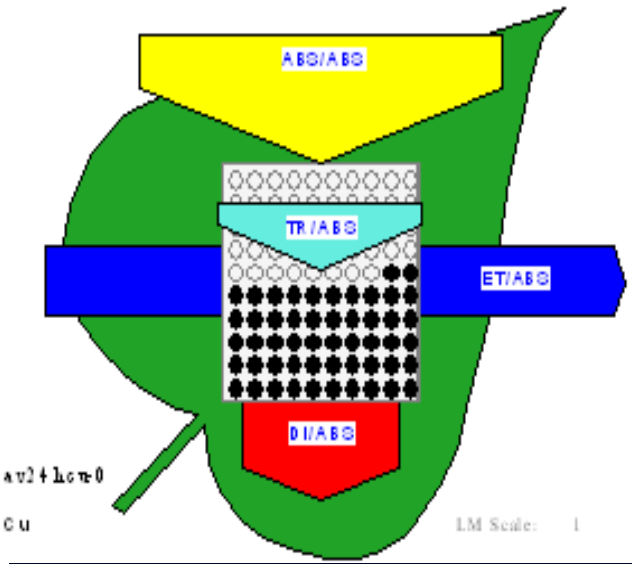
Heavy metals concentration (ppm)

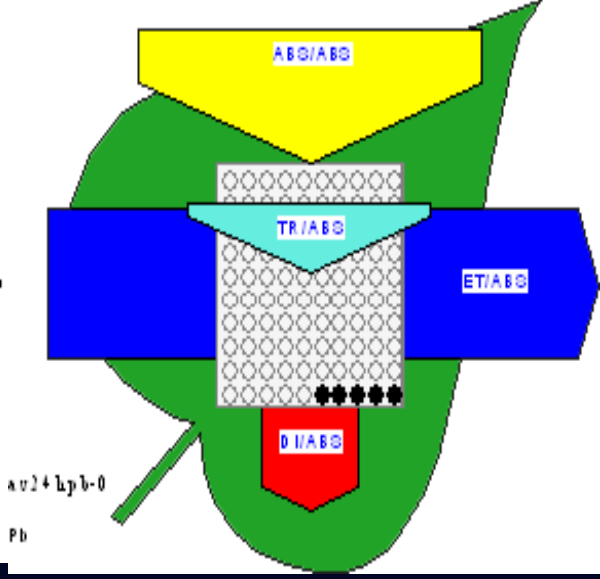
Maximal quantum efficiency of photosystem II (Fv/Fm)

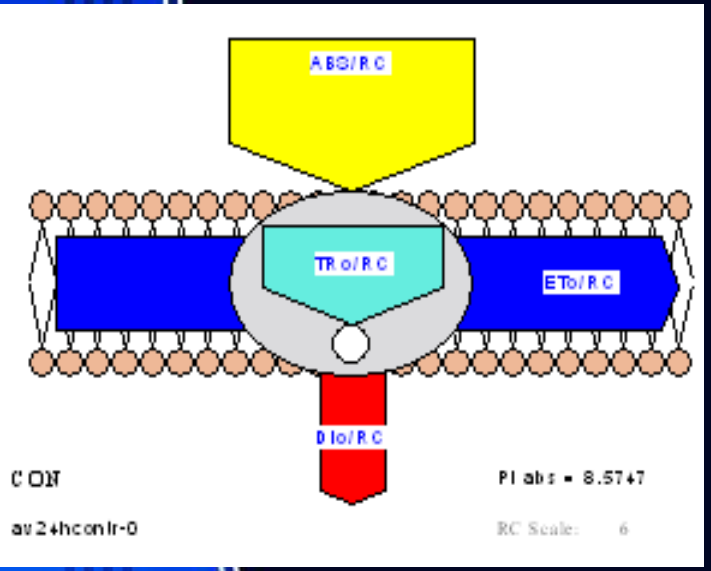


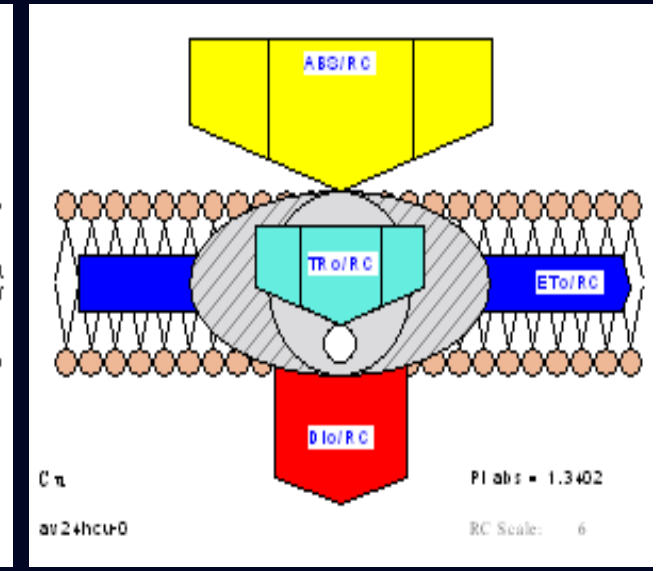
Heavy metals concentration (ppm)

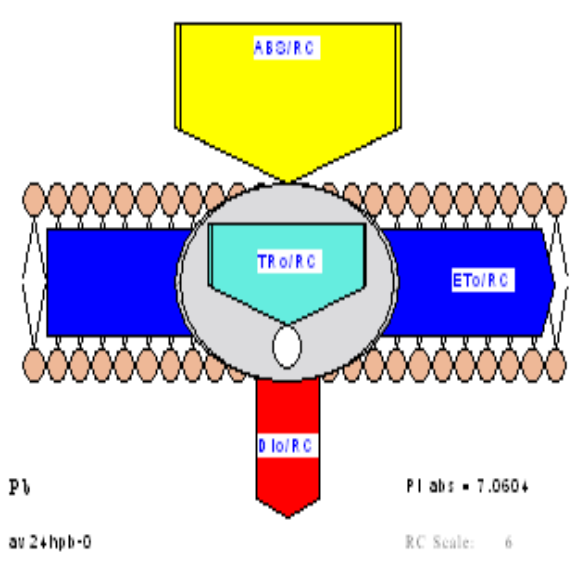

ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av24hconstr-0
CON
LM Scale: 1


ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av24hcu-0
Cu
LM Scale: 1

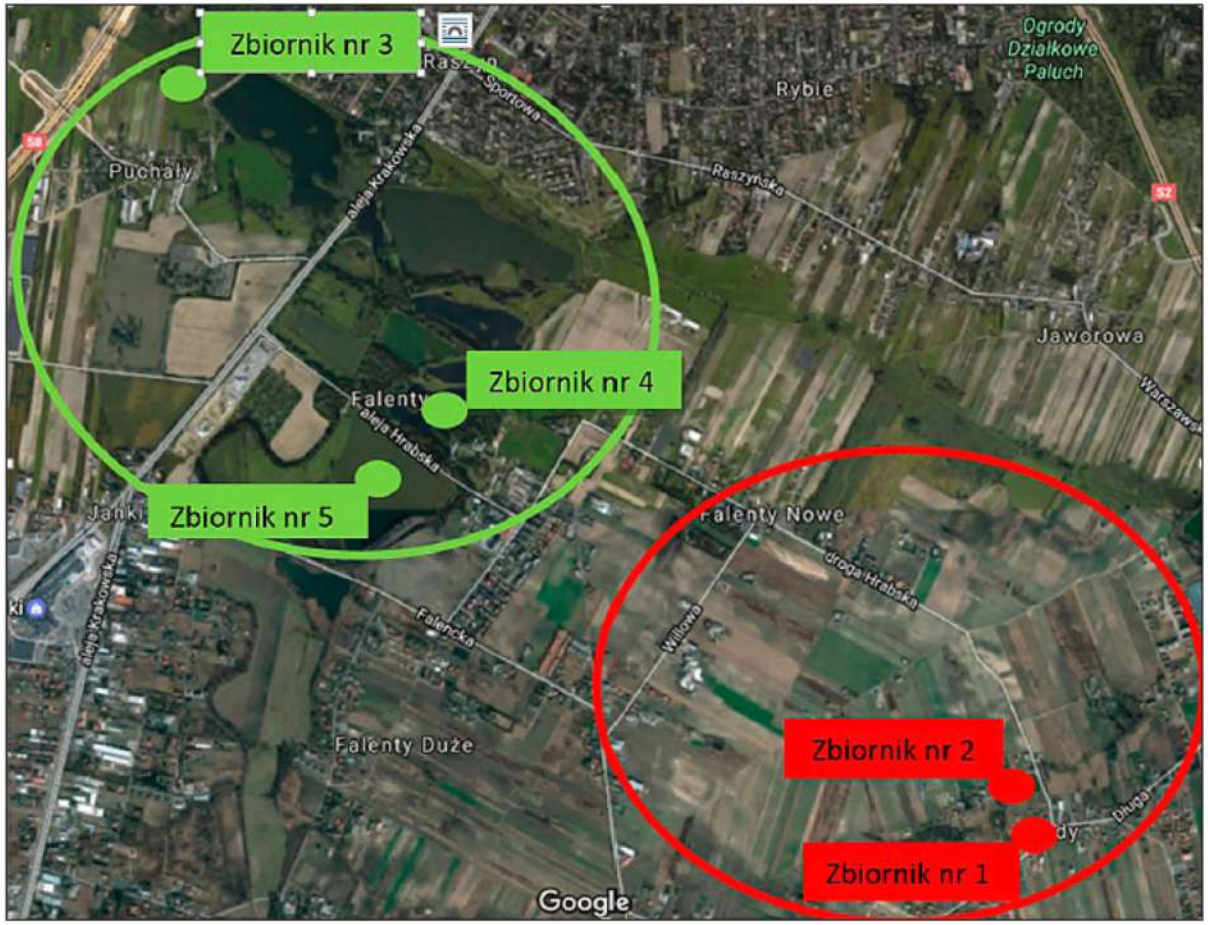

ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av24hpb-0
Pb


ABS/RC
TRo/RC
ETo/RC
DIo/RC
CON
Plabs = 8.5747
av24hconstr-0
RC Scale: 6


ABS/RC
TRo/RC
ETo/RC
DIo/RC
Cu
Plabs = 1.3402
av24hcu-0
RC Scale: 6

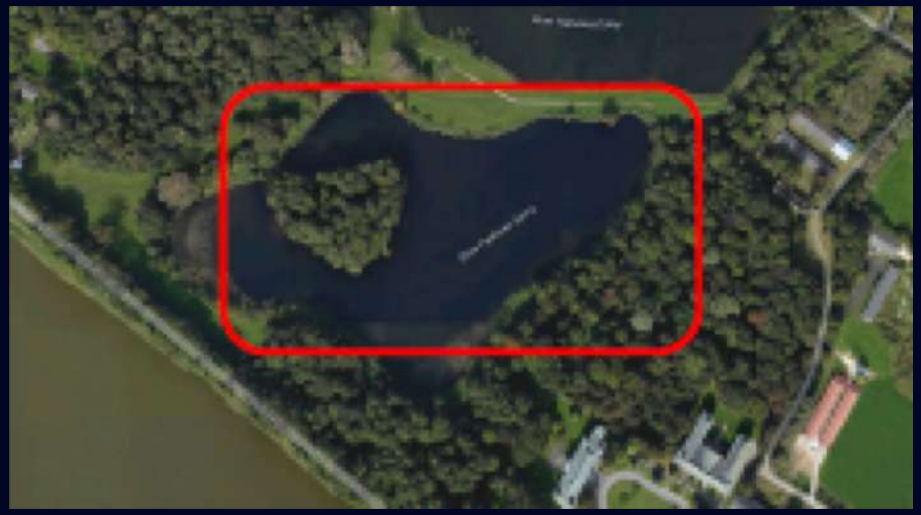

ABS/RC
TRo/RC
ETo/RC
DIo/RC
Pb
Plabs = 7.0604
av24hpb-0
RC Scale: 6

Nikodem Szymanski, Irena Burzyńska, Hazem Mohamed Kalaji, Grażyna Mastalerczuk. (2018) Fluorescencja chlorofilu jako narzędzie do oceny stopnia eutrofizacji ekosystemów wodnych na przykładzie stawów na obszarze gminy Raszyn. INŻYNIERIA EKOLOGICZNA 19, 2, 73-80.

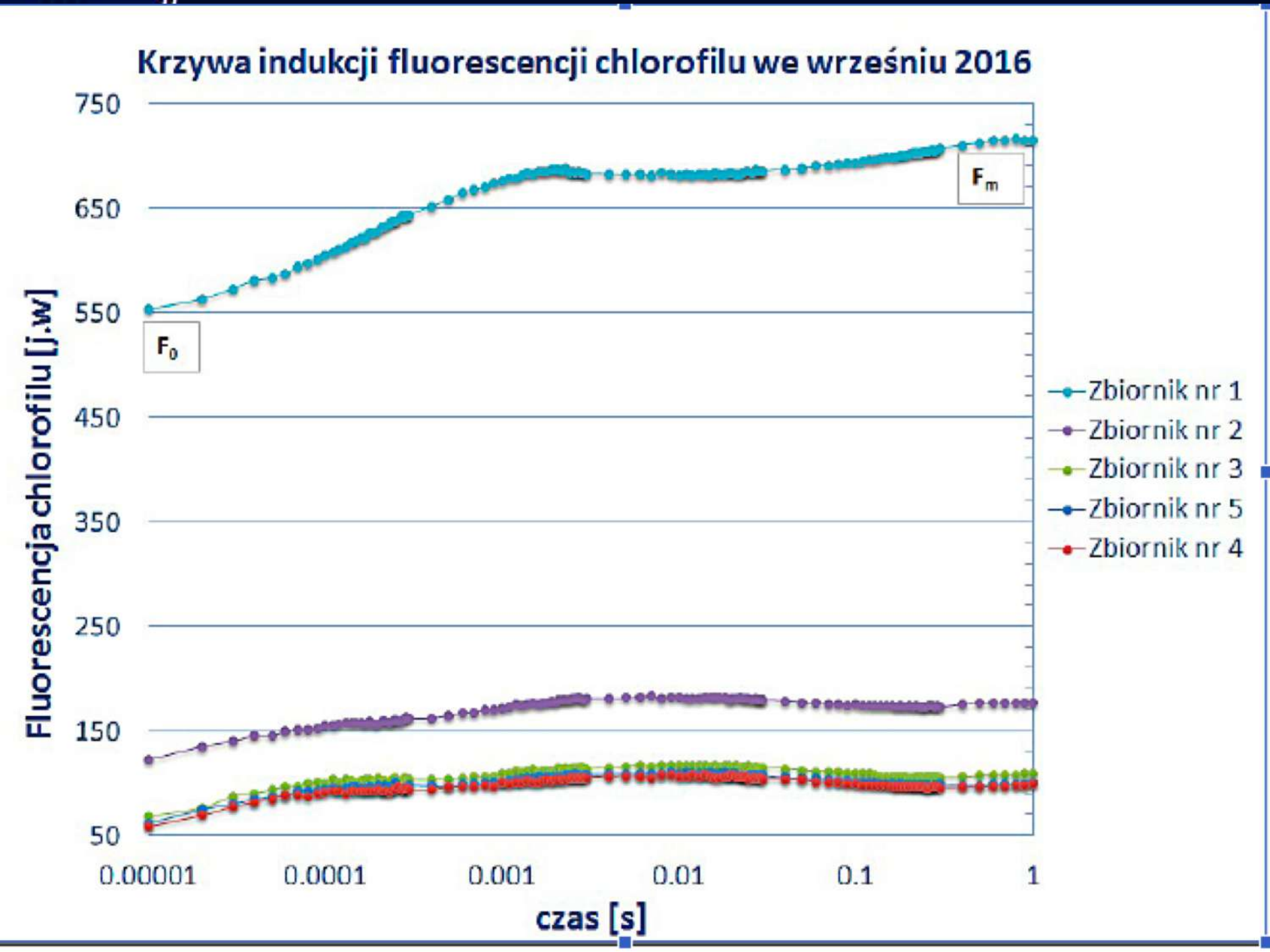


Rys. 1. Lokalizacja zbiorników wodnych położonych w województwie mazowieckim, w powiecie pruszkowskim w gminie Raszyn [maps.google.com]

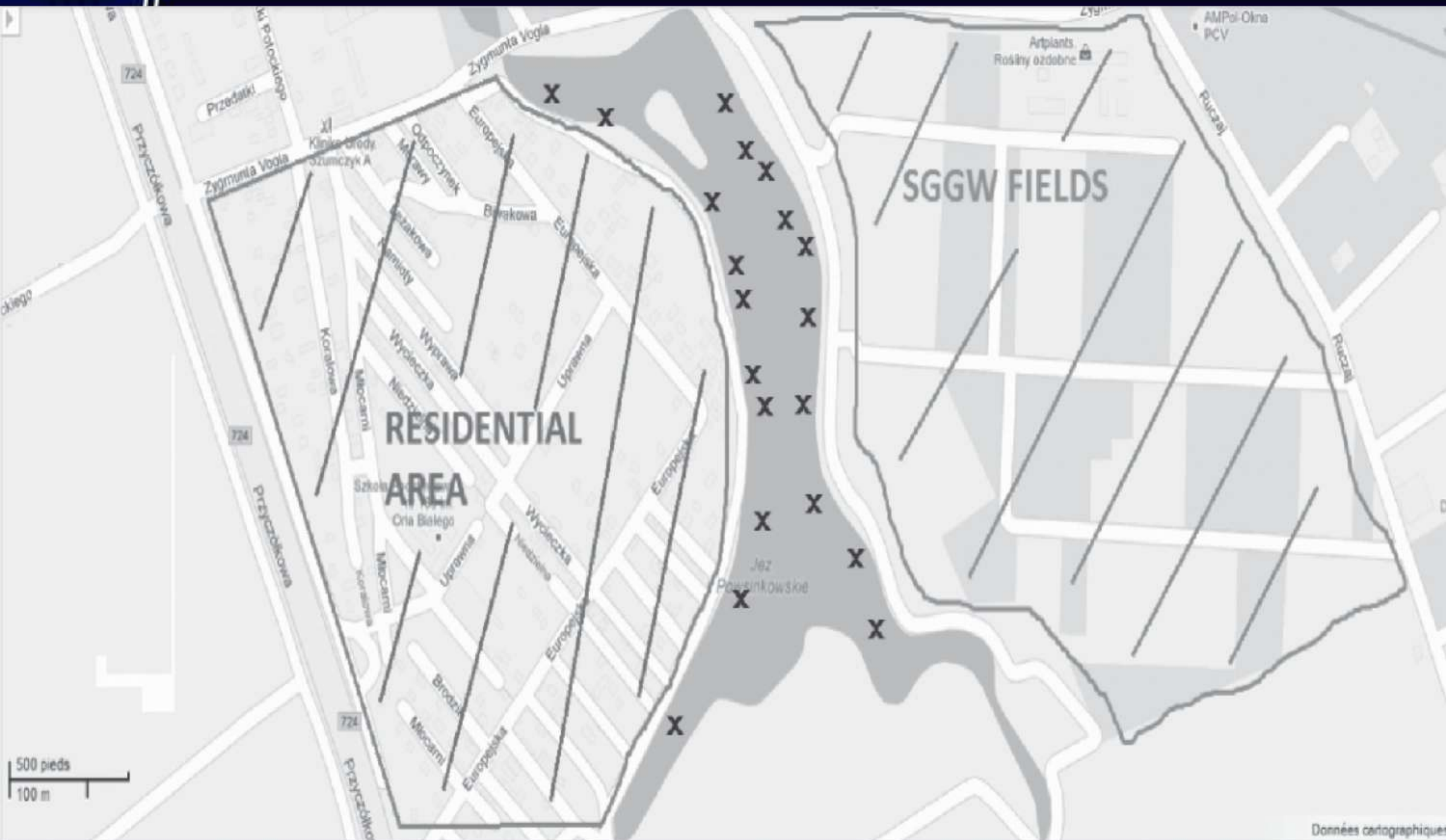
Fig. 1. Location of water reservoirs located in the Masovian Voivodeship, in the Pruszkow Poviat in the Raszyn commune [google.maps.com]



Handy PEA fluorimeter (Handy Plant Efficiency Analyzer) Hansatech Instruments Ltd.


Krzywa indukcji fluorescencji chlorofilu we wrześniu 2016
750
650
550
450
350
250
150
50
Fluorescencja chlorofilu [j.w]
F0
Fm
0.00001
0.0001
0.001
0.01
0.1
1
czas [s]
Zbiornik nr 1
Zbiornik nr 2
Zbiornik nr 3
Zbiornik nr 5
Zbiornik nr 4

Kalaji HM, Sytar O, Brestic M, Samborska IA, Cetner MD, Carpentier C. Risk Assessment of Urban Lake Water Quality Based on in-situ Cyanobacterial and Total Chlorophyll-a Monitoring. Polish Journal of Environmental Studies. 2016;25(2):655-661. doi:10.15244/pjoes/60895.


RESIDENTIAL AREA
SGGW FIELDS
Zygmunta Vogla
Przyczółkowa
Europejska
Ruczaj
AMPol-Okna PCV
Artplants Rośliny ozdobne
500 pieds
100 m
Données cartographiques

AlgaTorch bbe moldaenke - Germany

AlgaeTorch

The quick-and-easy portable measuring instrument. Switch on, dip in, read off!

- Fast and simple algae monitoring
- Designed for all types of surface water: lakes, reservoirs, rivers and bathing water

	Maximum total chlorophyll-*a* level observed in individual samples (µg/L)	
Date	Eastern (SGGW fields) lake side	Western (residential) lake side
24 May	41	57
31 May	28	36
6 June	24	52
14 June	66	137
20 June	37	35
27 June	40	41
3 July	21	29
11 July	38	65
18 July	47	71
25 July	44	93

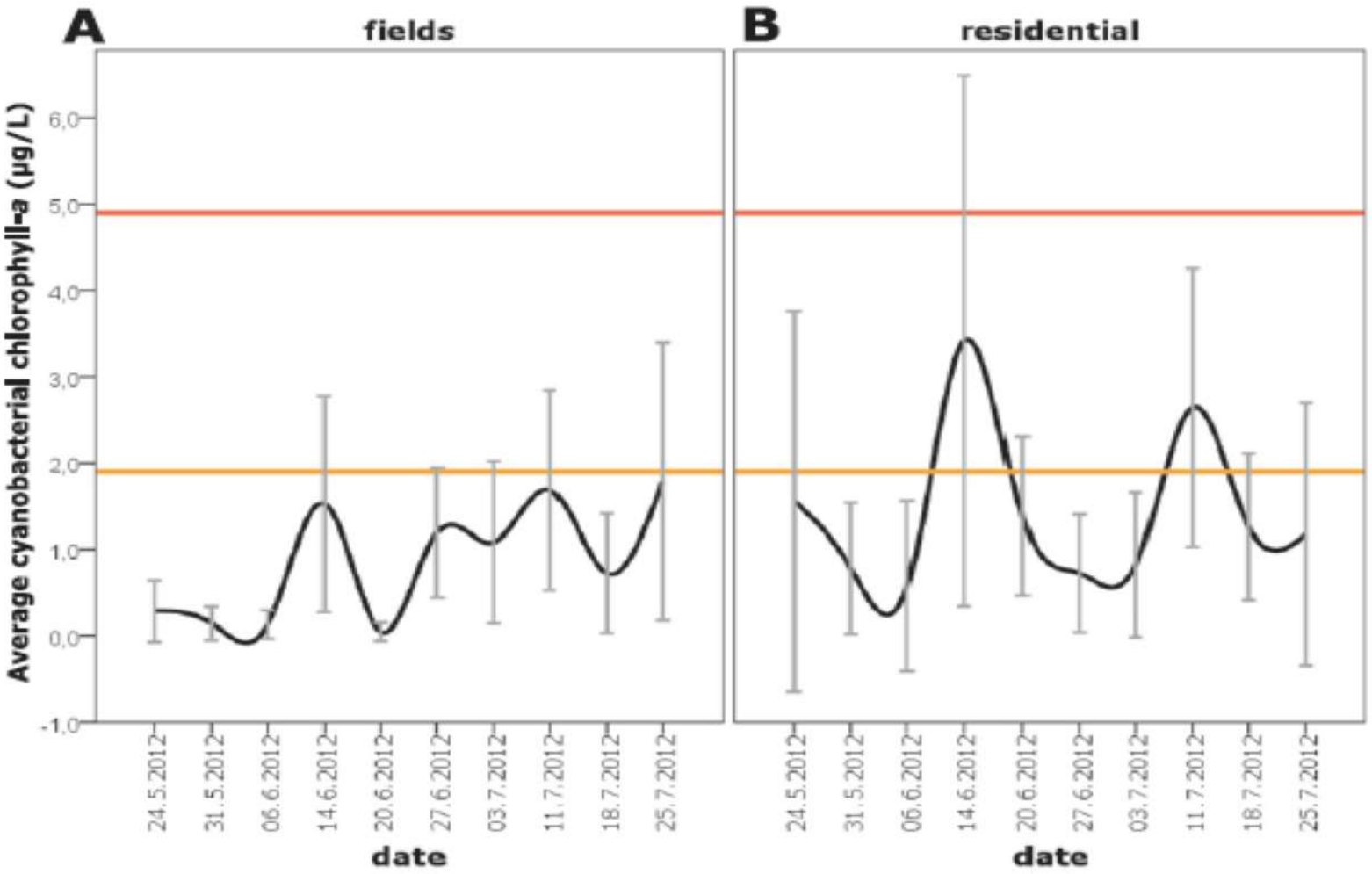


Fig. 4. Results of average cyanobacterial chlorophyll-a related to Alert Level 1 (1.9 µg/L cyanobacterial chl.-*a*, yellow line) and Alert Level 2 (4.9 µg/L cyanobacterial chlorophyll-a, red line) for the WULS–SGGW side ("fields"; (A)) and the residential side (B) of Lake Powsinkowskie.

The Dream

Stress Identifying

IF:	
Tf(max)	280 - 294
Area	> 37968.00
PHI(Po)	0.79 - 0.77
Kn	< 0.58
Kp	< 2.13
ABS/RC	> 2.48
TRo/RC	1.81 - 1.95
ETo/RC	> 1.13
DIo/RC	< 0.53
PI	>20.47

Water Stress

IF:	
Tf(max)	290 - 299
Area	37968.00
PHI(Po)	0.79 - 0.77
Kn	0.58 – 0.66
Kp	< 2.13
ABS/RC	< 2.48
TRo/RC	1.81 - 1.95
ETo/RC	1.13- 1.25
DIo/RC	< 0.53
PI	20 - 30

Nitrogen deficiency

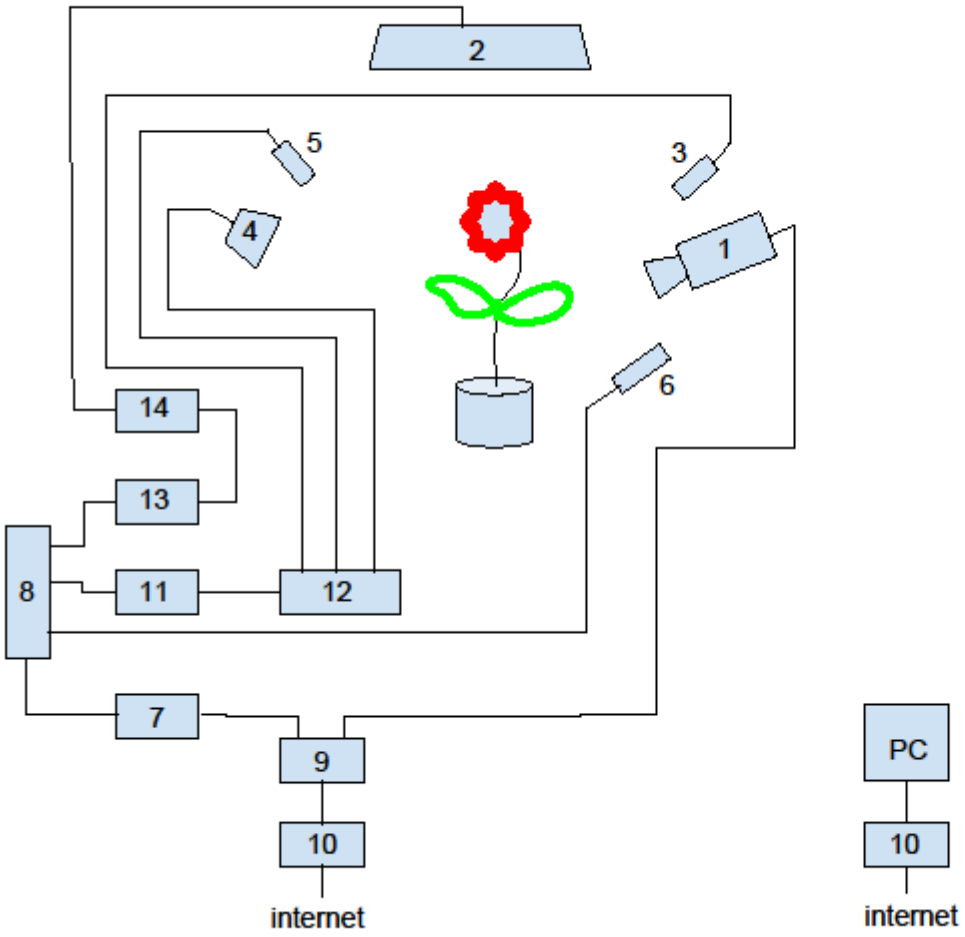
Instrument Development

I am Thirsty.

Please give me water

Stress identification

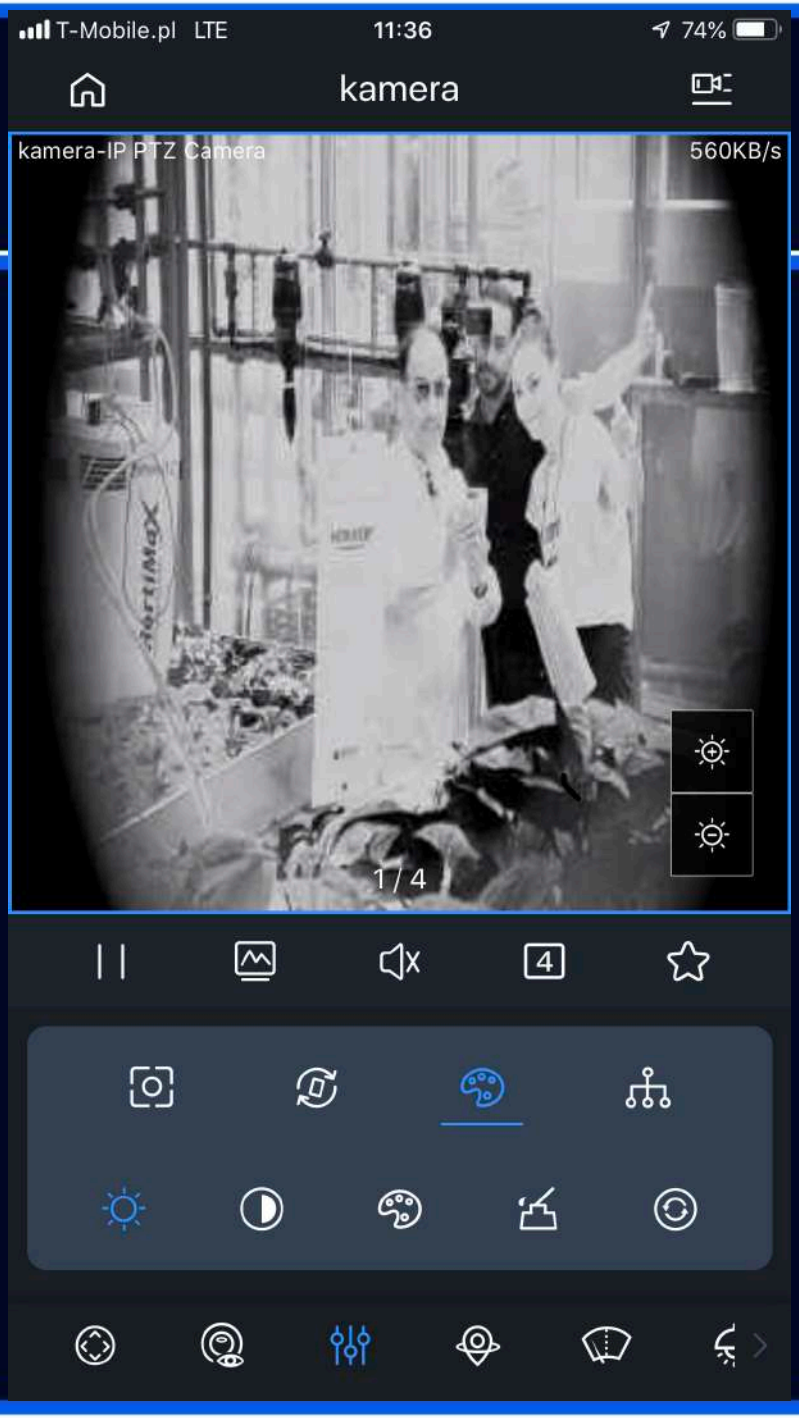
Biological feedback system



1. fluorescence detector radiometer CMOS camera
2. greenhouse actinic light LED lamp
3. fluorescence induction blue laser
4. RGB measurement light LED lamp
5. red laser
6. light meter
7. Raspberry pi microcomputer Python libraries and scripts for controllers and interfaces
8. USB hub
9. ethernet switch
10. internet router
11. USB-DMX512 interface
12. DMX512 PWM on-off controller
13. USB-DALI (Digital Addressable Lighting Interface)
14. DALI light controller-dimmer

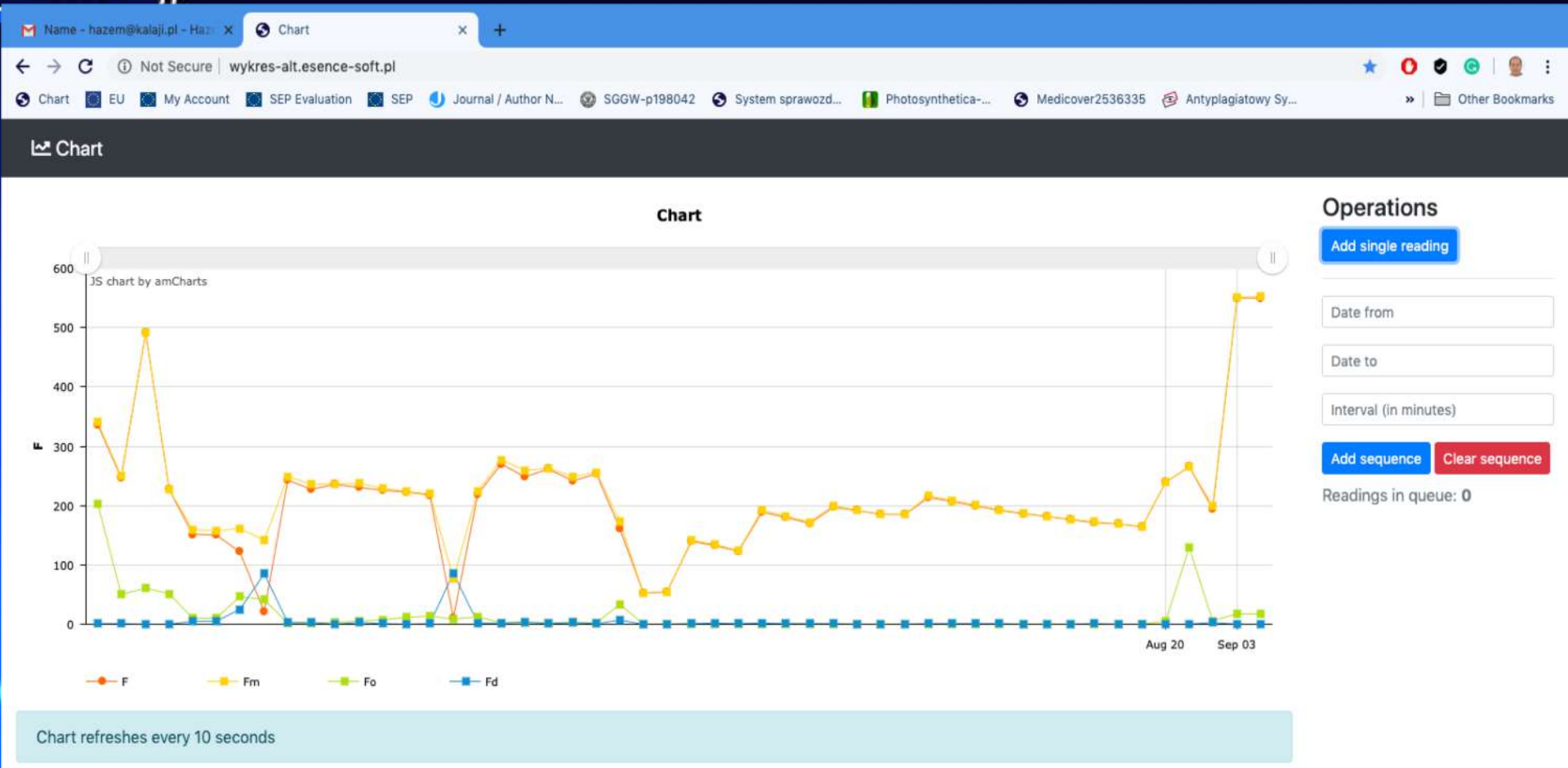
PC remote computer vision (openCV) data acquisition and control algorithms


TVP INFO
teleexpress
EXTRA
WIDZIANE
Z
POLSKI
00:08:53 | 00:17:36


T-Mobile.pl LTE
11:36
74%
kamera
kamera-IP PTZ Camera
560KB/s
1 / 4

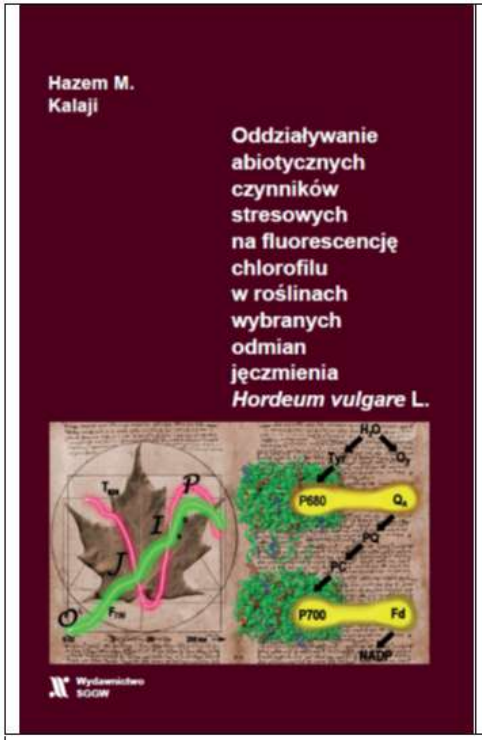
Continuous measurements

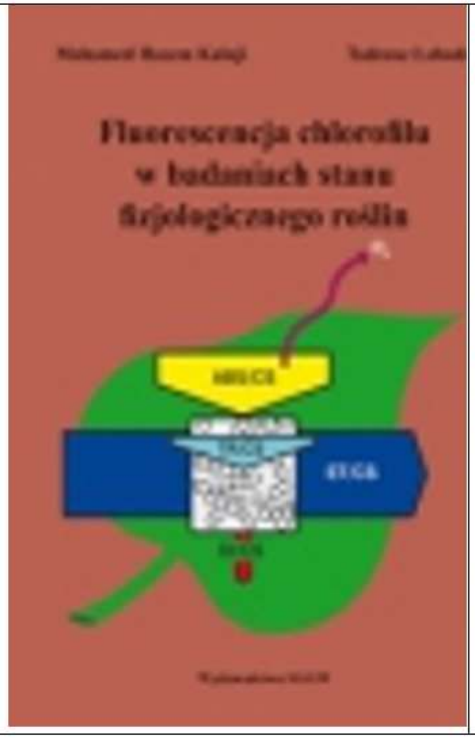
http://wykres-alt.esence-soft.pl/



Rewitalizacja Rotundy PKO Banku Polskiego

(Pracownia architektoniczna Gowin & Siuta, kraków)


Hazem M.
Kalaji
Oddziaływanie abiotycznych czynników stresowych na fluorescencję chlorofilu w roślinach wybranych odmian jęczmienia *Hordeum vulgare* L.
P680
P700
Fd
NADP
Wydawnictwo SGGW


Fluorescencja chlorofilu w badaniach stanu fizjologicznego roślin


Misurare la vitalità delle piante per mezzo della fluorescenza della clorofilla
FILIPPO BUSSOTTI, MOHAMED HAZEM KALAJI, ROSANNA DESOTGIU, MARTINA POLLASTRINI, TADEUSZ ŁOBODA, KAROLINA BOSA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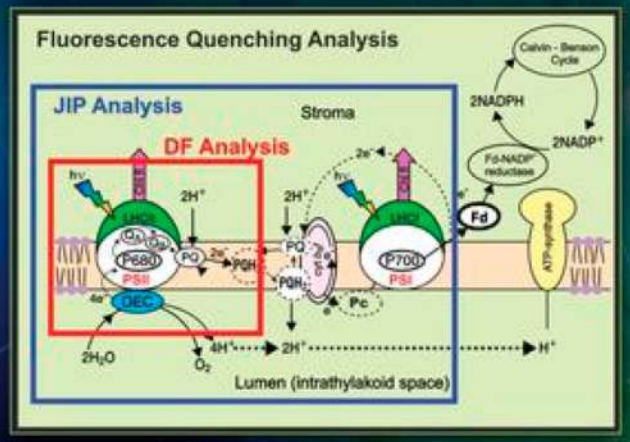
Chlorophyll Fluorescence

Understanding Crop Performance — Basics and Applications



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