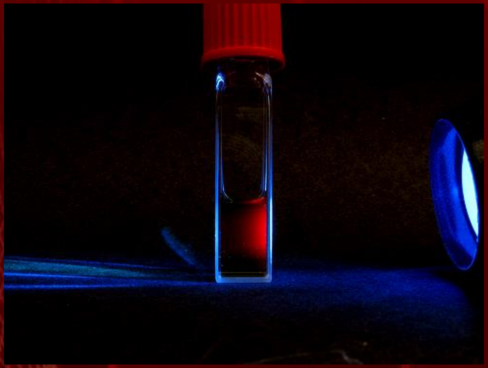
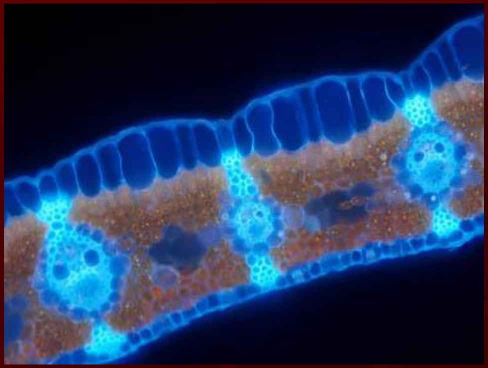
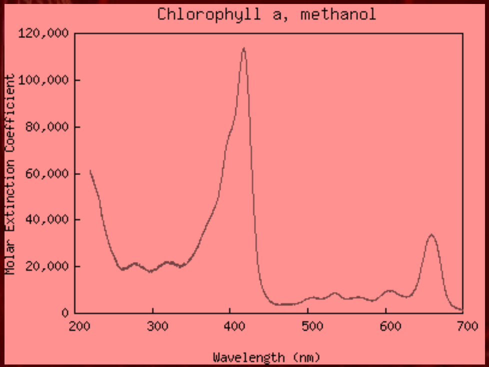
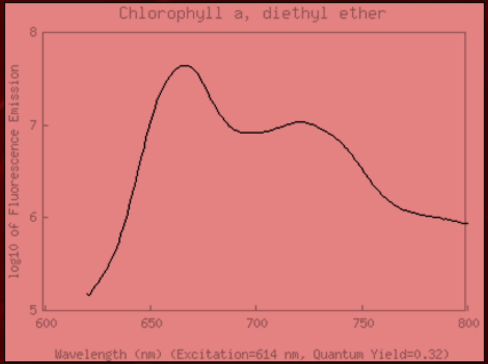
Applications of Chlorophyll Fluorescence in Agricultural Sciences



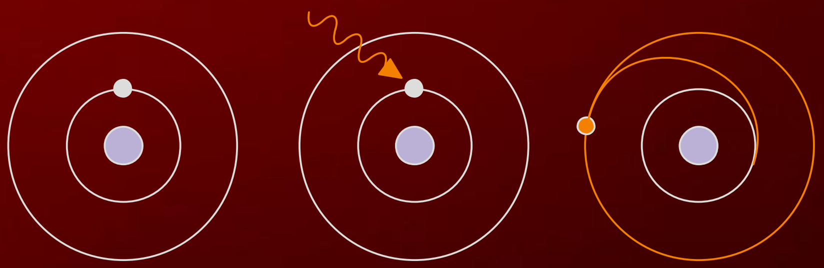






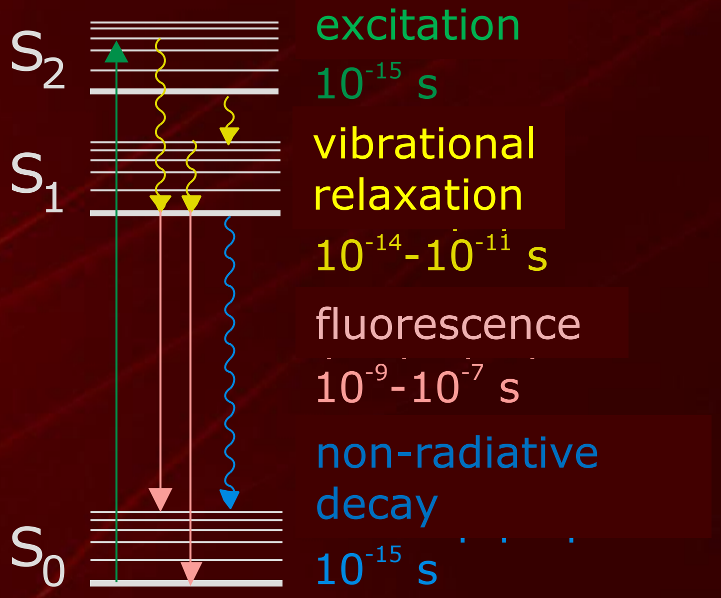
Georgios Liakopoulos – gliak@aua.gr

Radiation absorption by certain molecules causes temporary changes in energy content of a suitable electron. Electron transition results in orbital shift

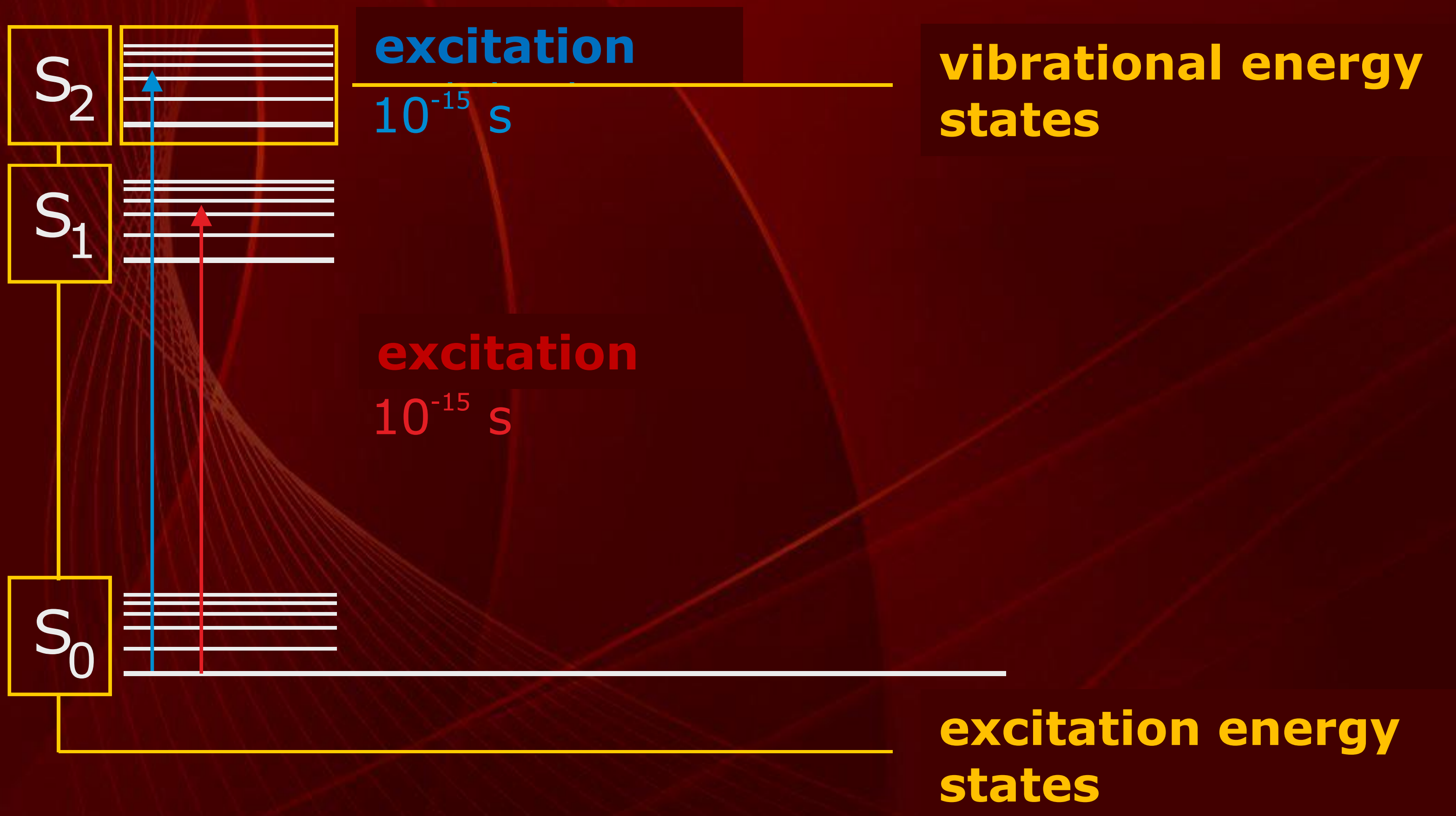


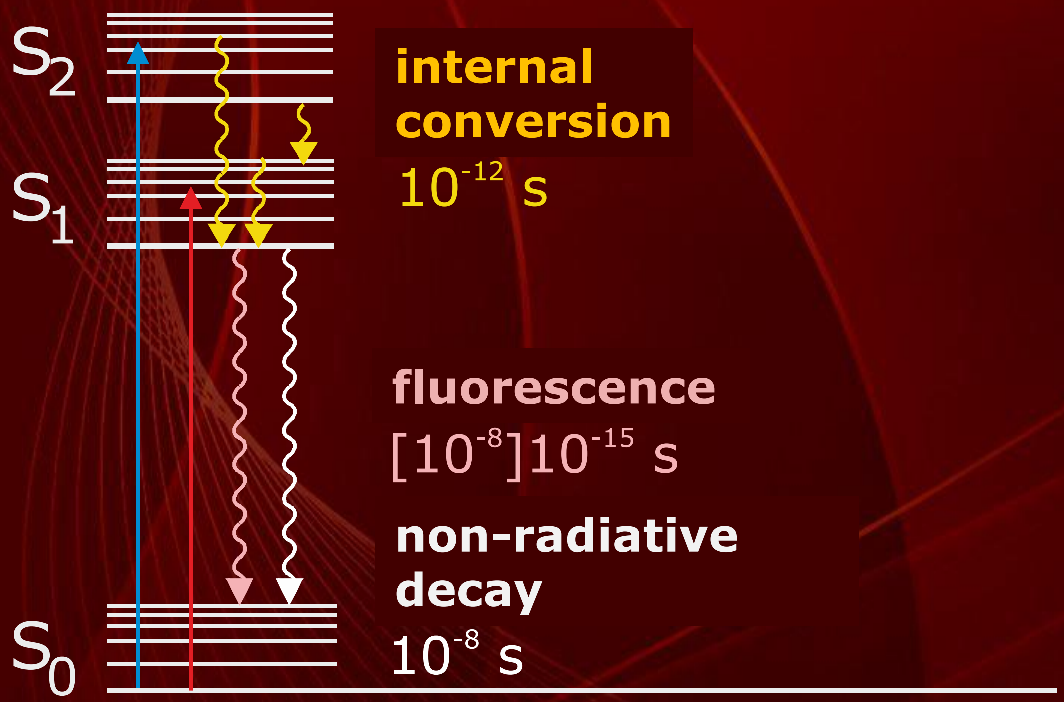
Return of the electron to its fixed orbital is done by simultaneous energy transmission

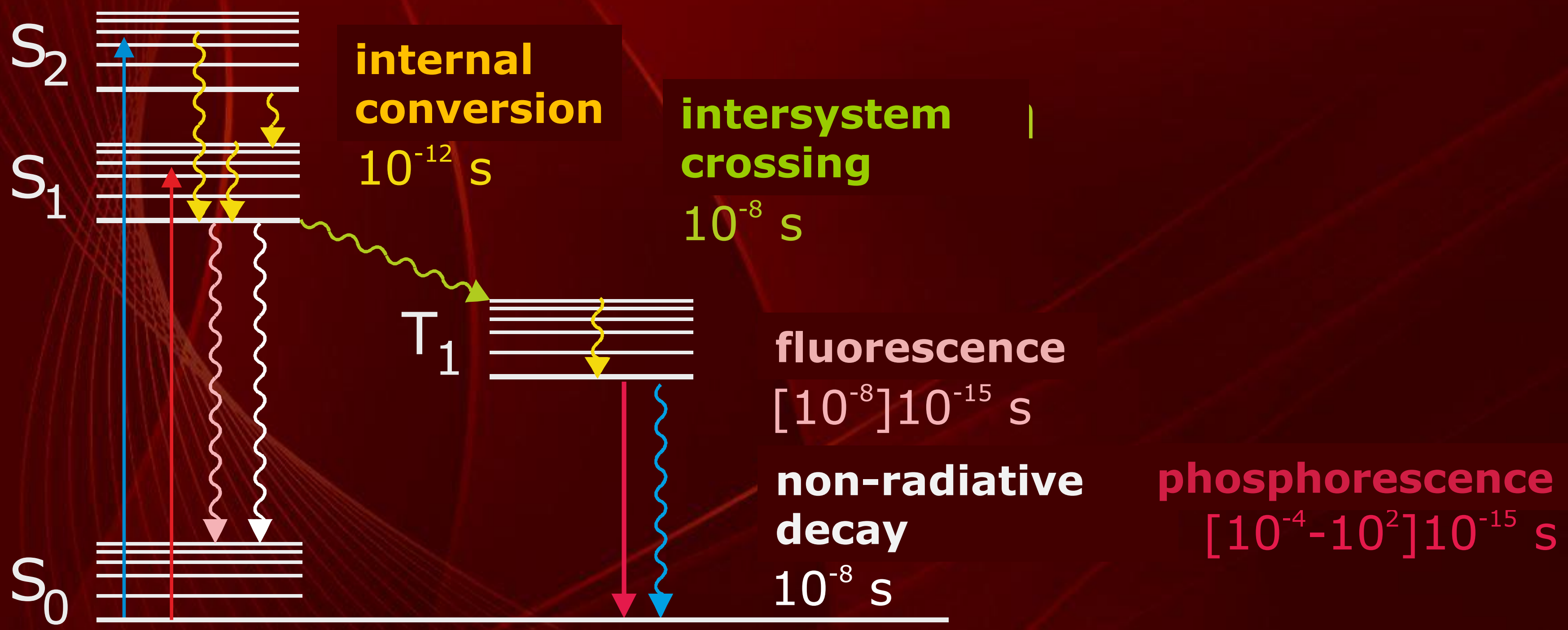
Energy transmission can be done in many ways: fluorescence emission, thermal dissipation, etc.



Fluorescence




S2
S1
S0
internal conversion
10^{-12} s
fluorescence
[10^{-8}]10^{-15} s
non-radiative decay
10^{-8} s


S2
S1
S0
T1
internal conversion
10-12 s
intersystem crossing
10-8 s
fluorescence
[10-8]10-15 s
non-radiative decay
10-8 s
phosphorescence
[10-4-102]10-15 s

Fluorescence

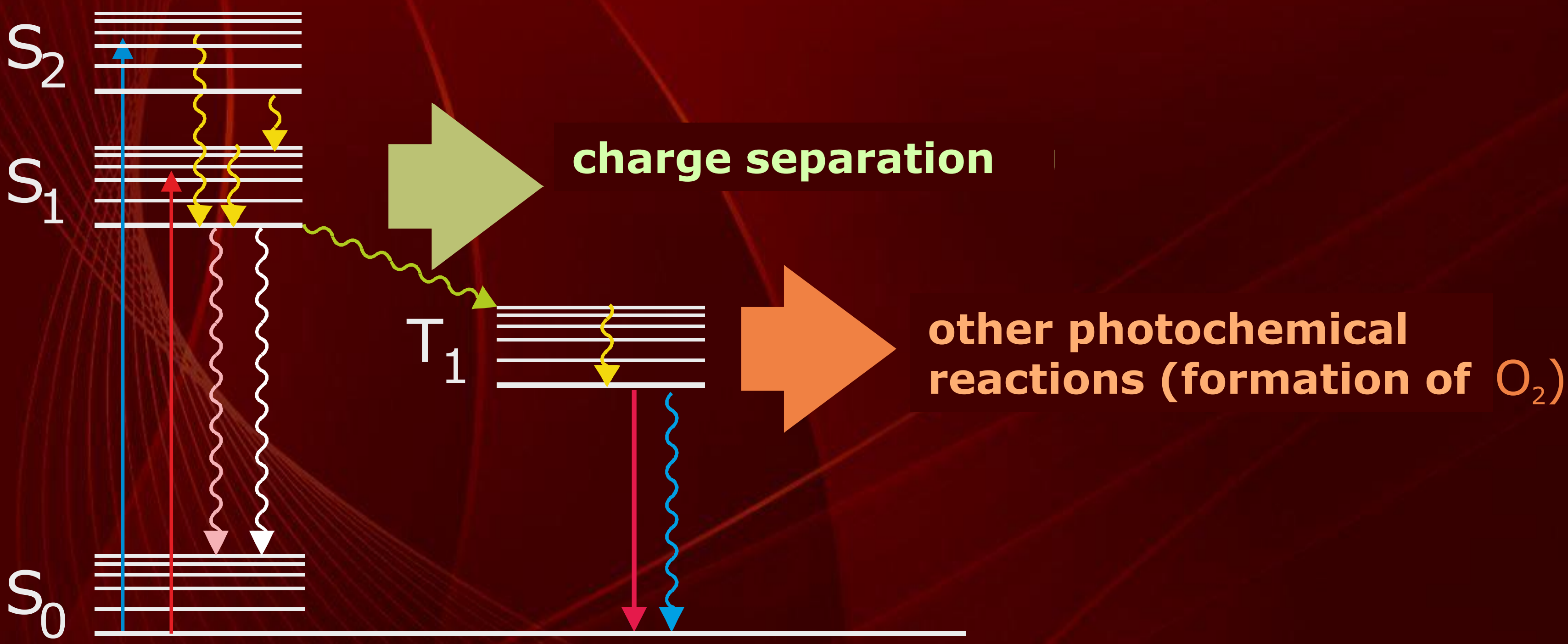
internal conversion 10^{-12} s

intersystem crossing 10^{-8} s

fluorescence $[10^{-8}]10^{-15}$ s

non-radiative decay 10^{-8} s

phosphorescence $[10^{-4}-10^{2}]10^{-15}$ s



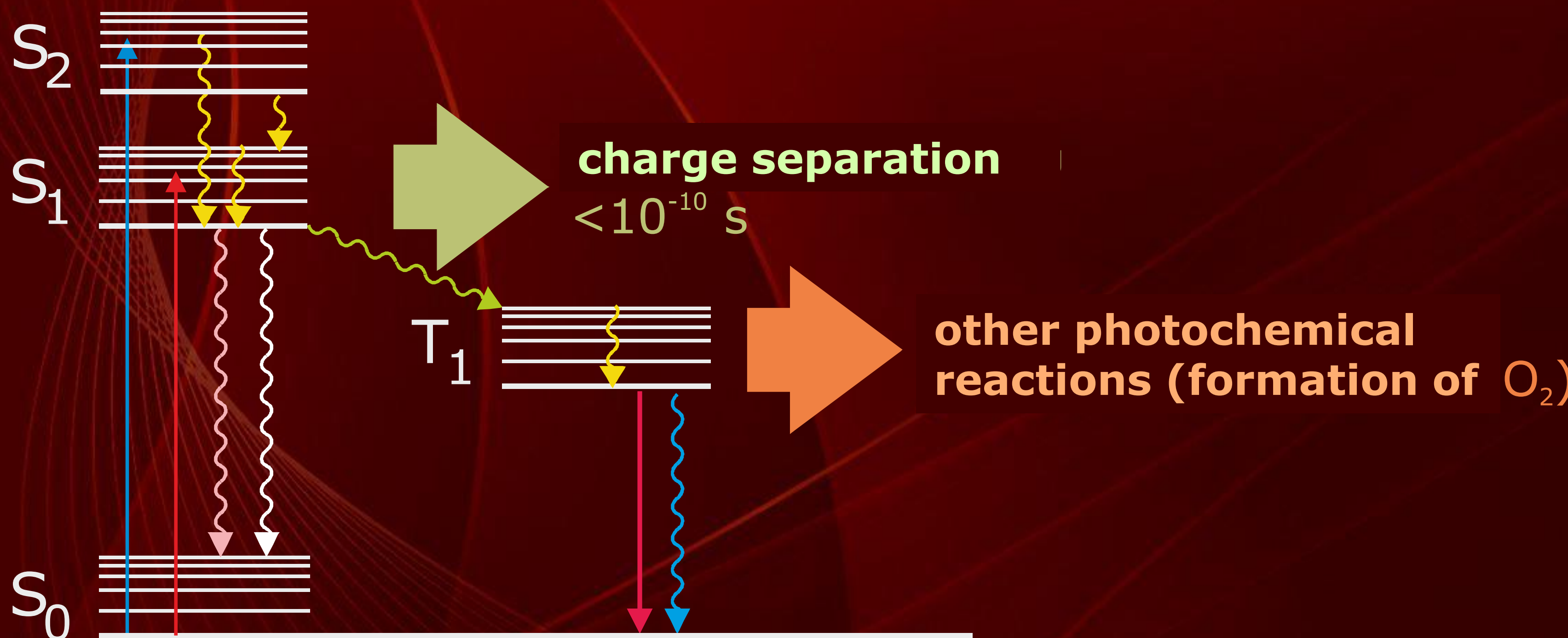
internal conversion 10^{-12} s

intersystem crossing 10^{-8} s

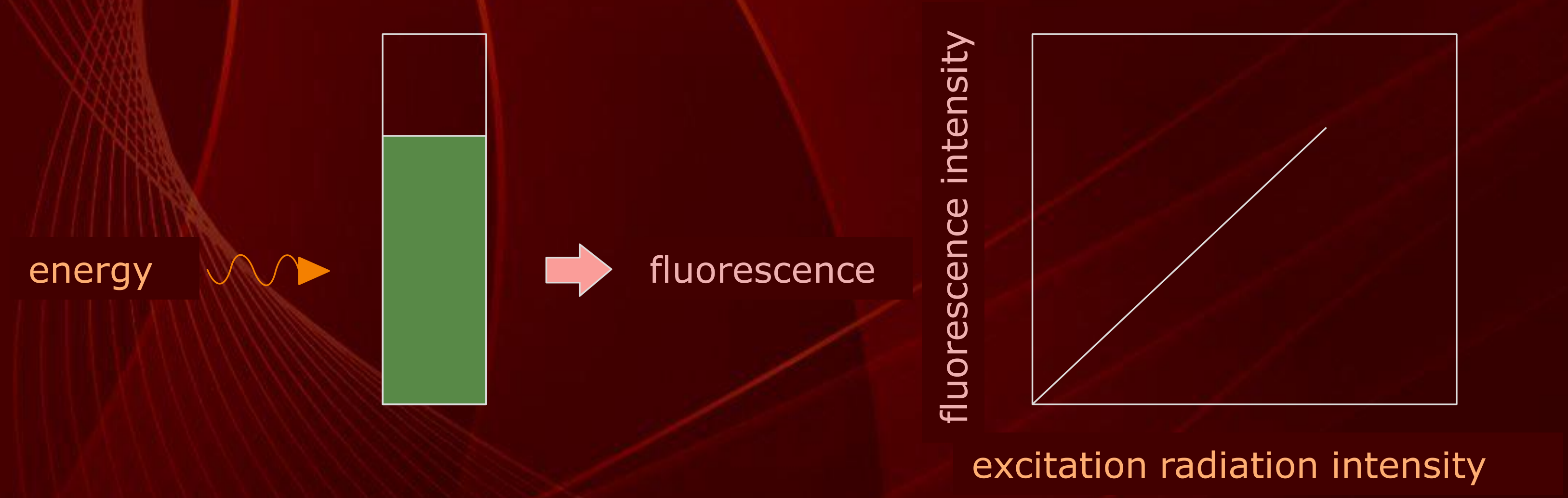
fluorescence $[10^{-8}]10^{-15}$ s

non-radiative decay 10^{-8} s

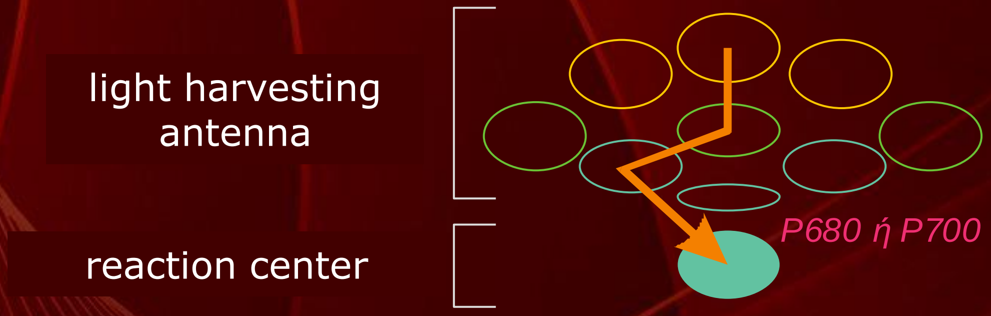
phosphorescence $[10^{-4}-10^{2}]10^{-15}$ s



In a solution of chlorophyll (*in vitro*) intensity of emitted fluorescence is linearly dependent with intensity of excitation radiation. I.e. fluorescence yield, FY is stable.

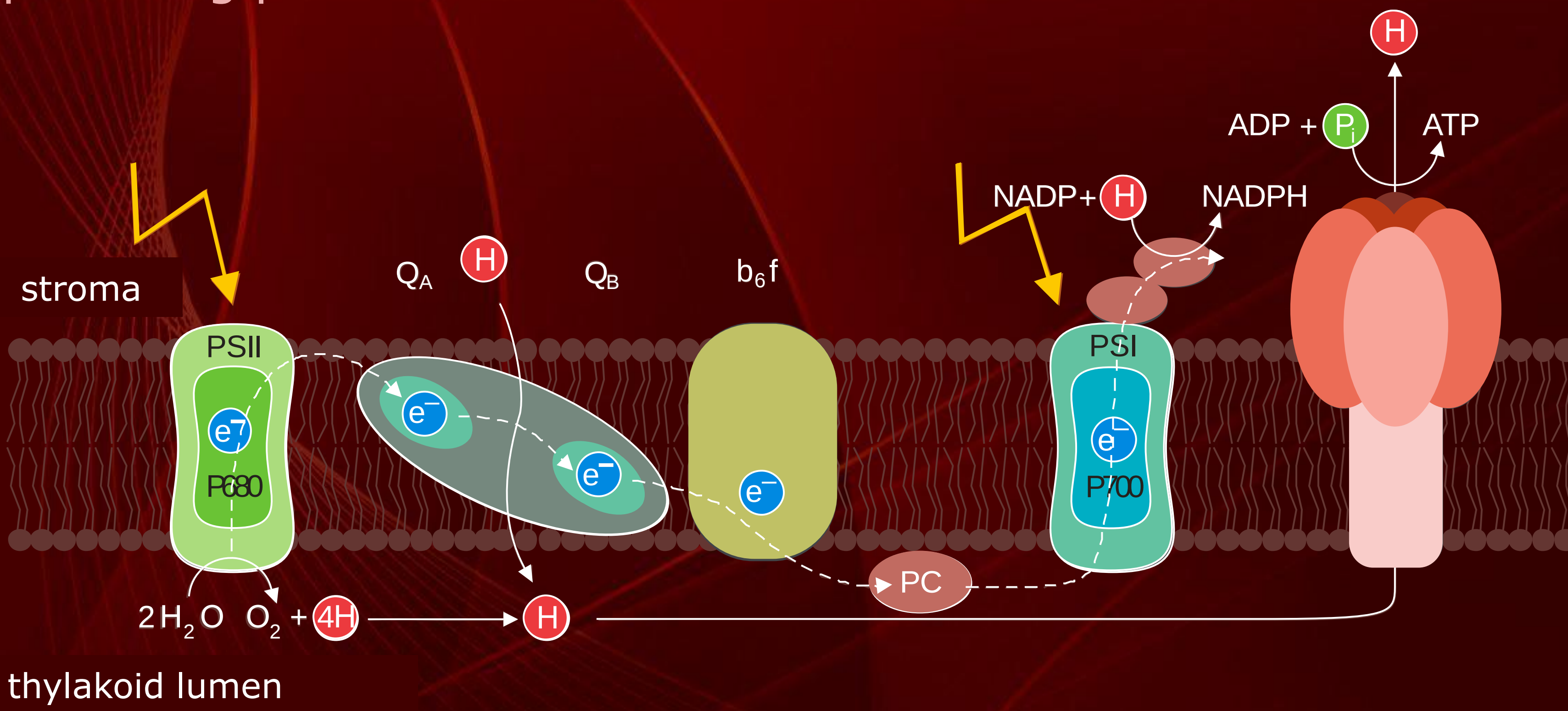


In the photosynthetic apparatus *(in vivo)* chlorophyll molecules are located in the two photosystems PSII και PSI and associated light harvesting complexes. In this environment, FY is not stable. Changes in FY can be interpreted photochemically (e.g. they provide information about the function of photochemical reactions of photosynthesis).



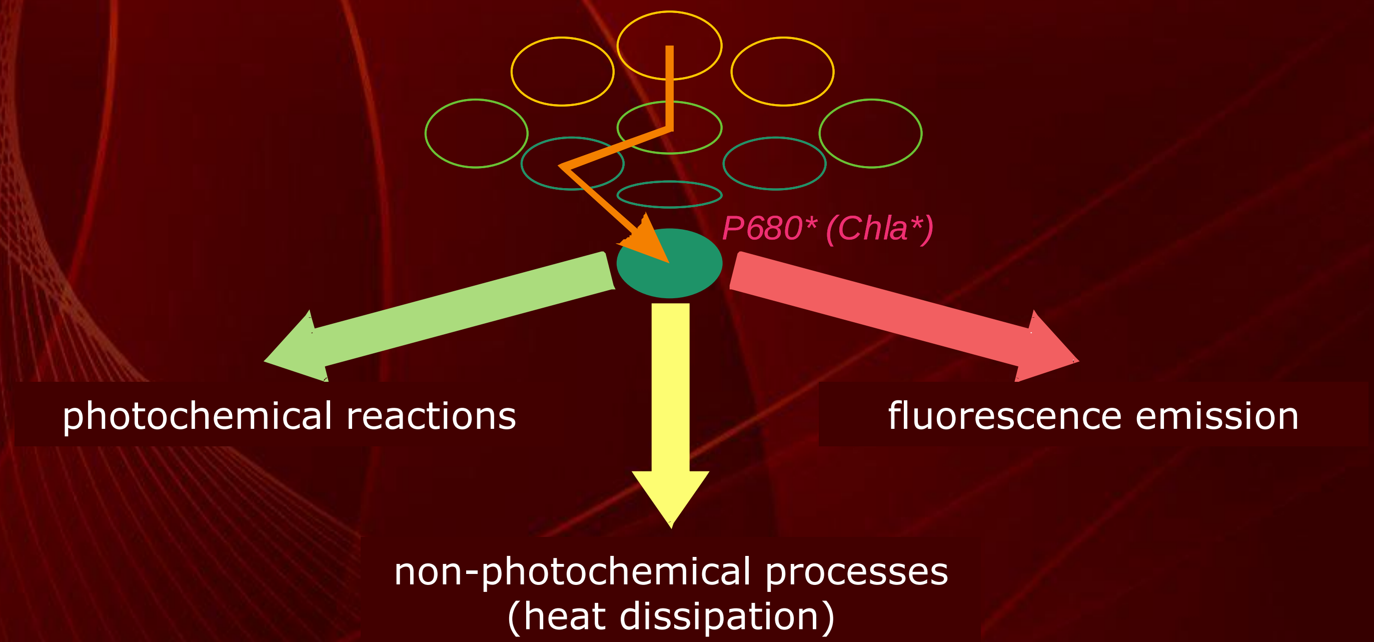
Photosystems and Photochemical Reactions

Photon absorption by the photosystems results in photochemical electron flow. However, photochemical quenching of energy may saturate when energy flow is high. In this case, the contribution of non-photochemical energy quenching processes increases.



An excited chlorophyll molecule of the photosystem II may

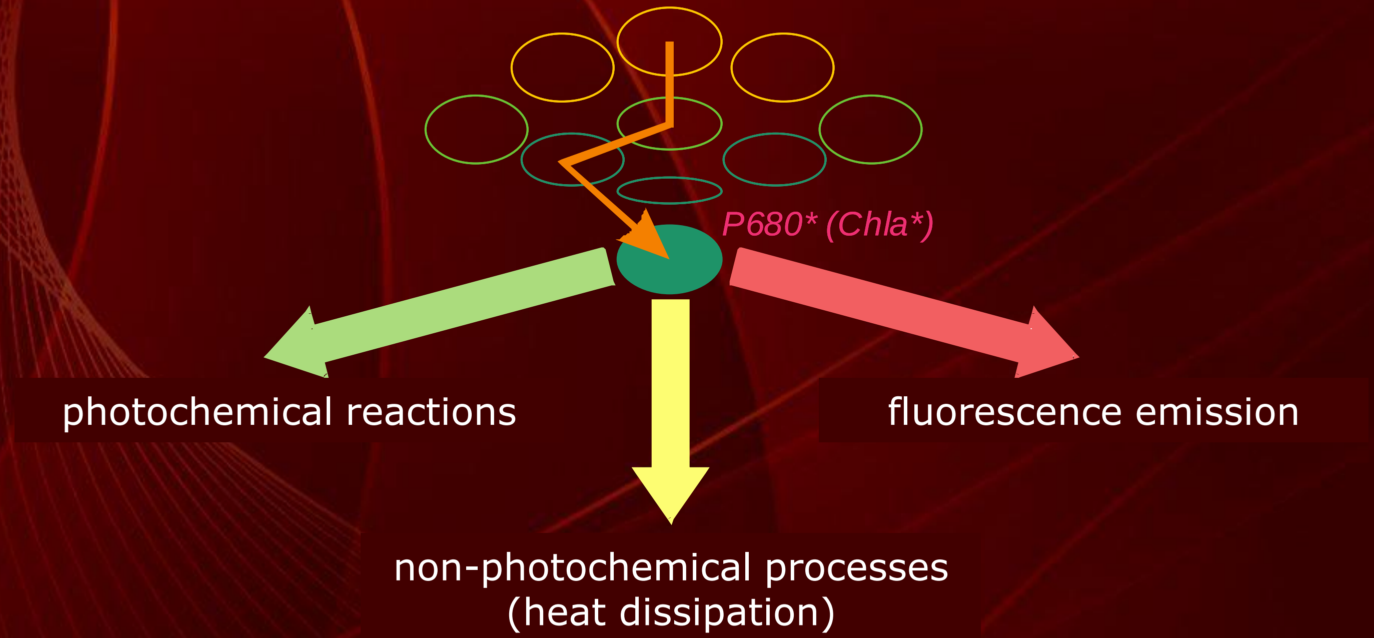
loose an electron and be oxidized (qP)



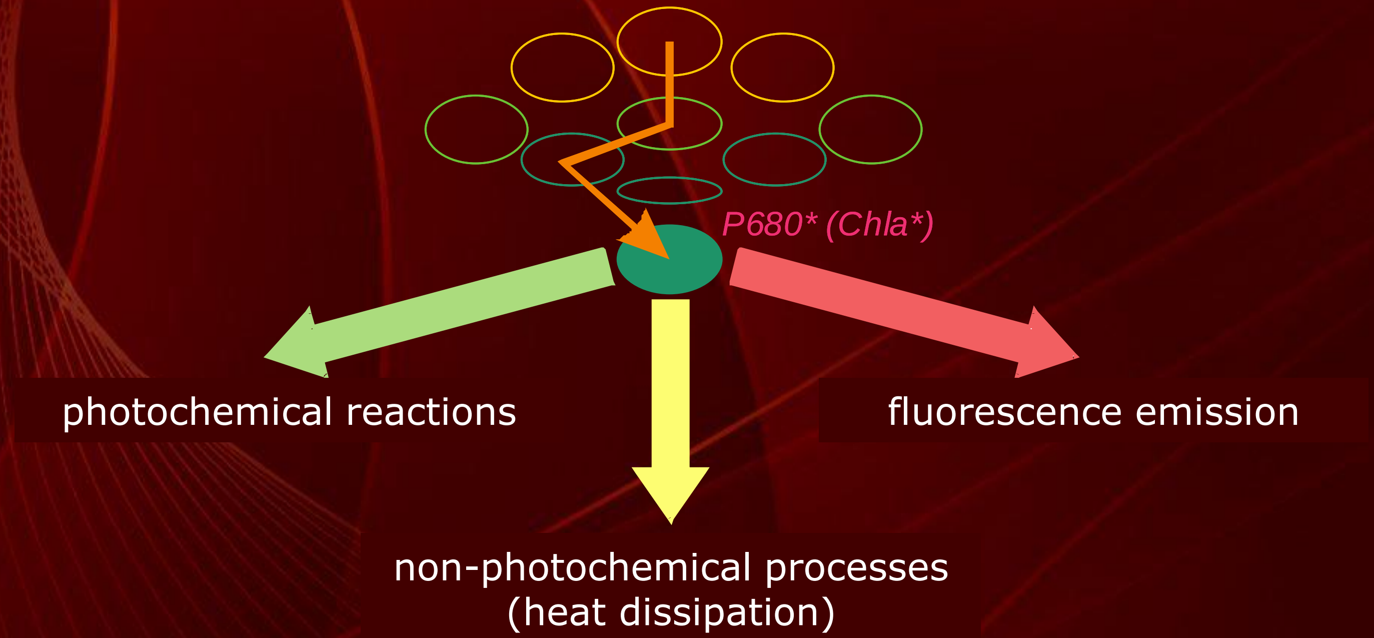
Chlorophyll Fluorescence and Other Quenching Processes

An excited chlorophyll molecule of the photosystem II may

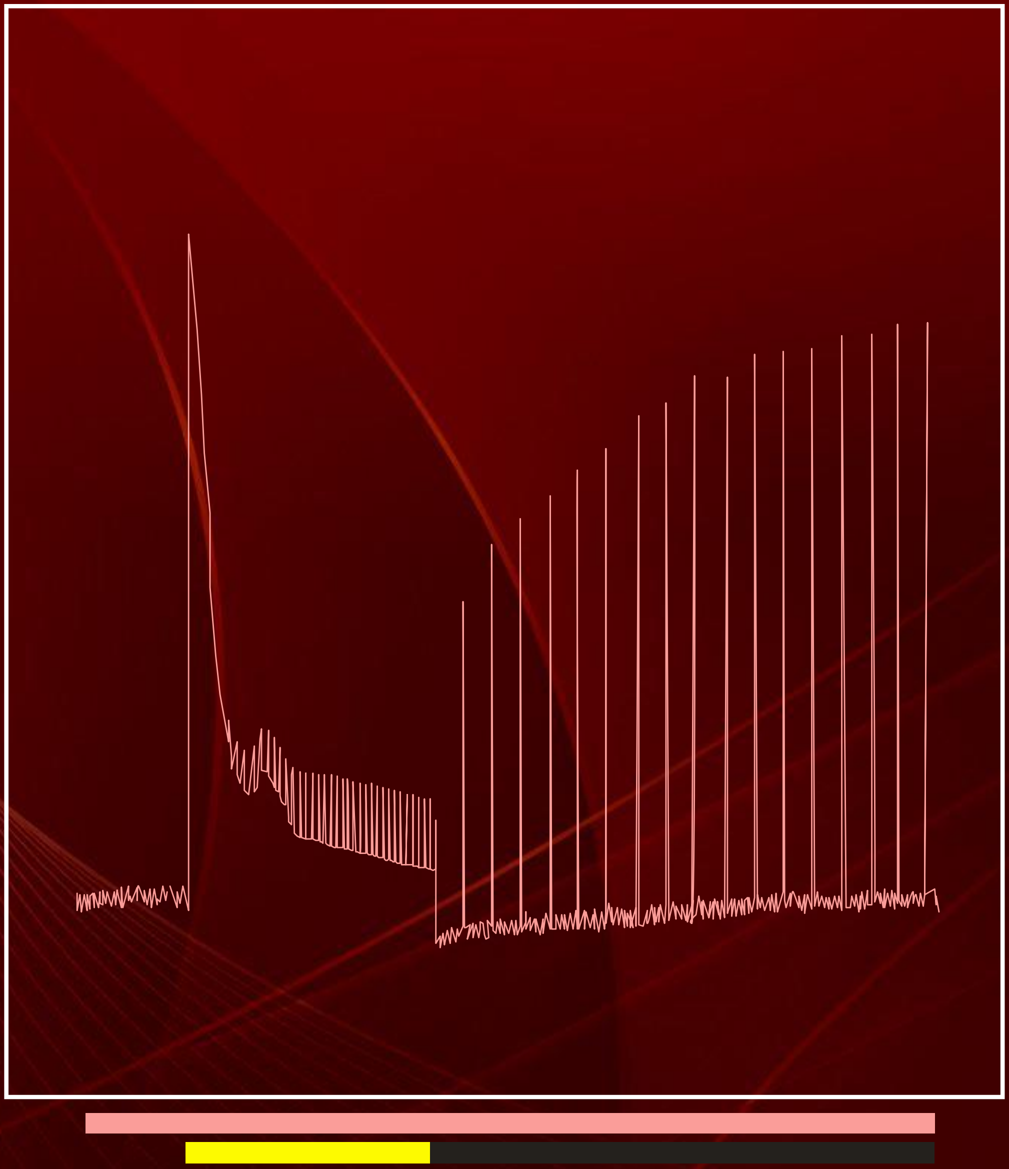
quench energy through thermal dissipation (qN or NPQ)



An excited chlorophyll molecule of the photosystem II may quench energy through fluorescence emission



Chlorophyll Fluorescence: *a signature of photosynthesis*



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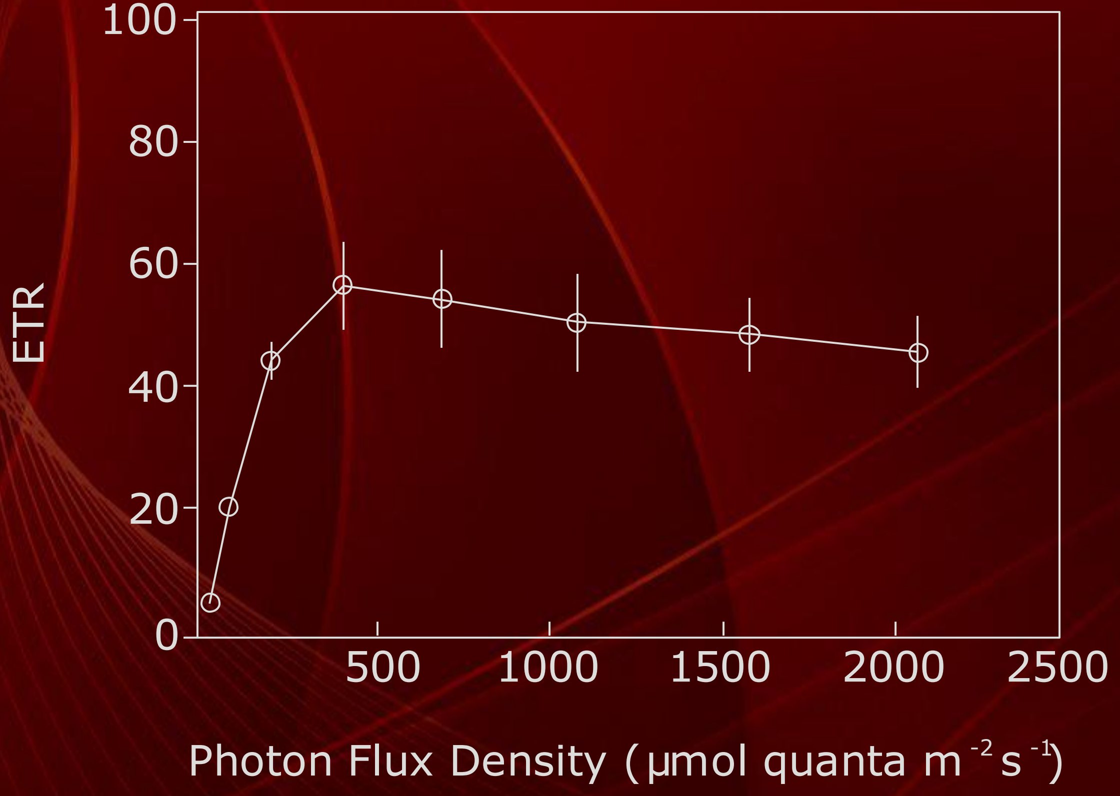

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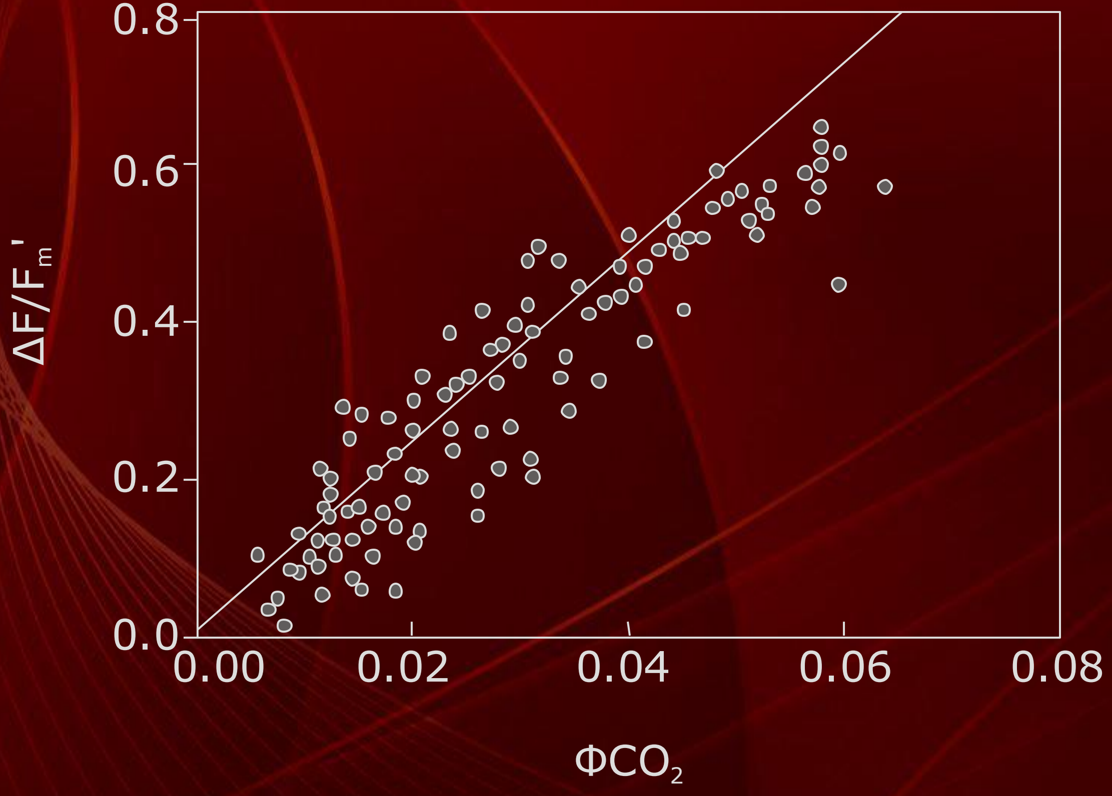
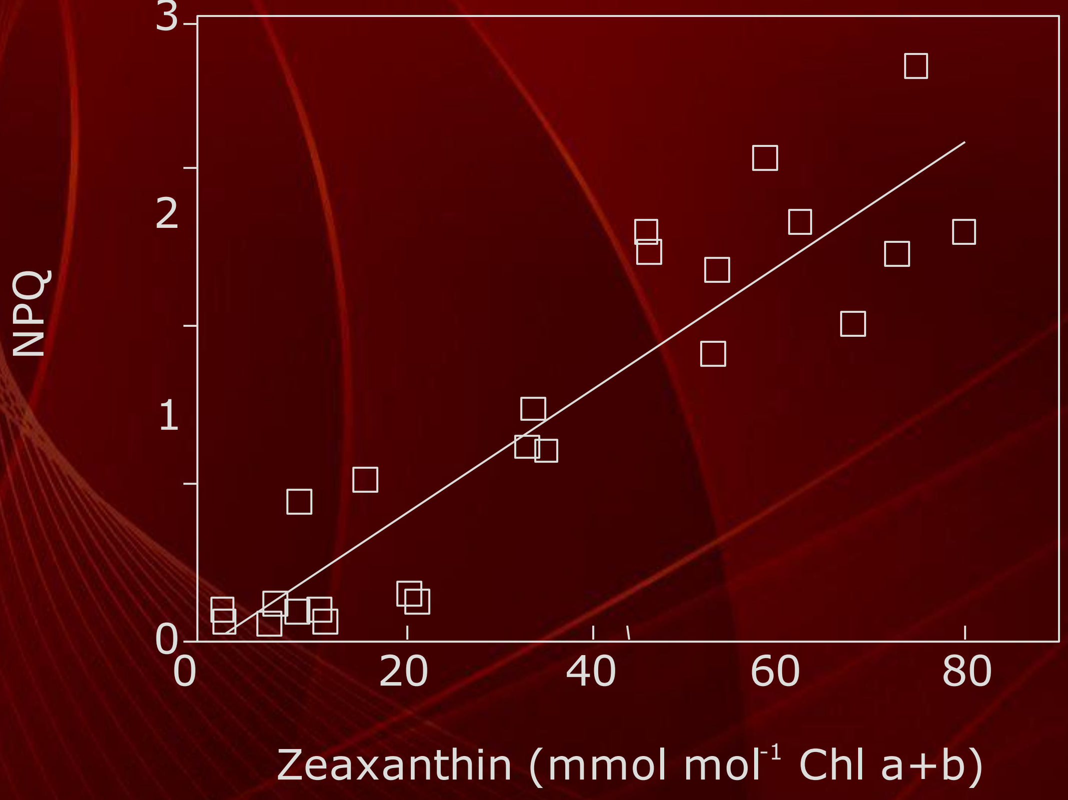



3
2
1
0
NPQ
0
20
40
60
80
Zeaxanthin (mmol mol-1 Chl a+b)

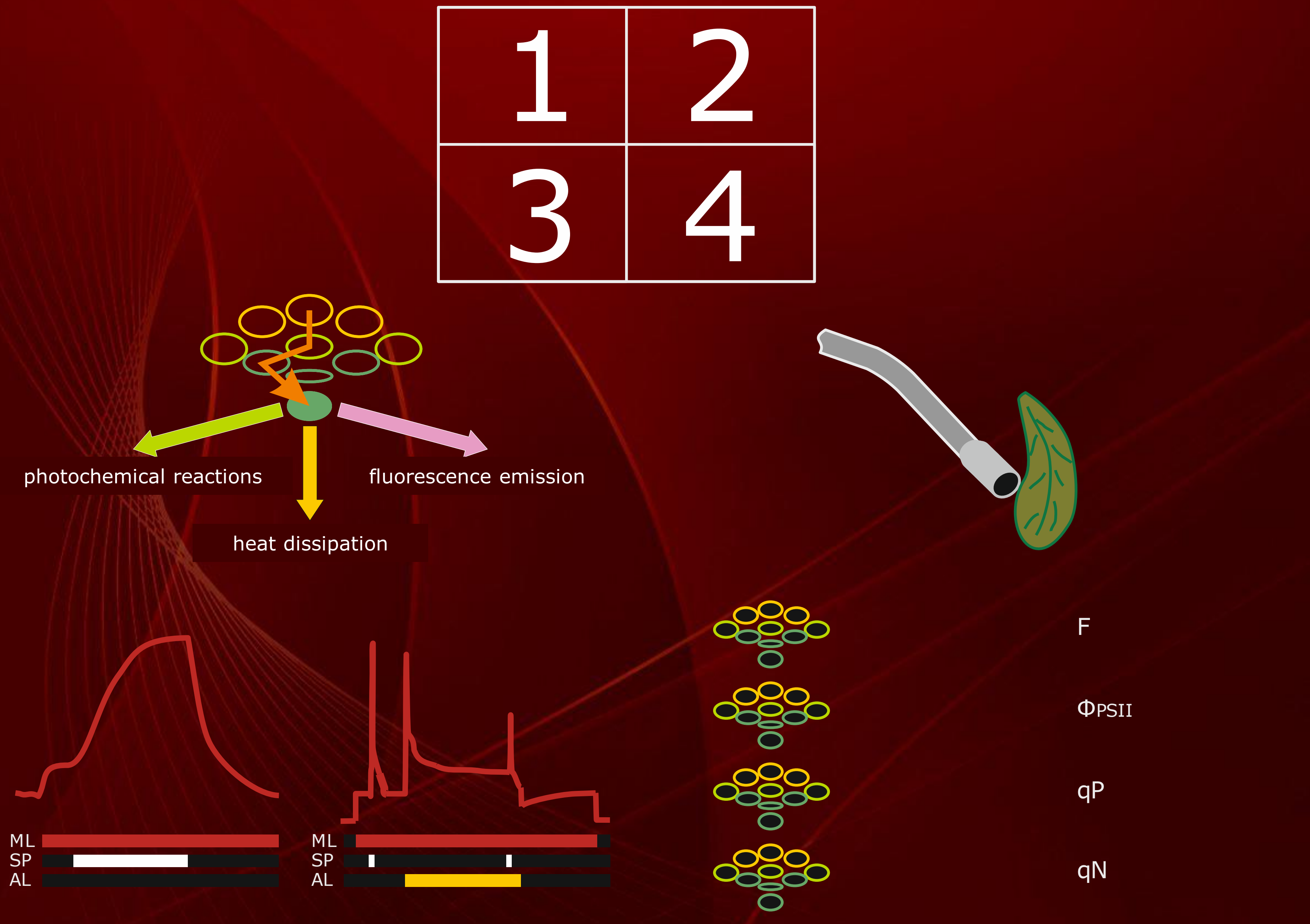
Advantages of the Chlorophyll Fluorescence Technique

- Rapid measurements
- High sensitivity
- A non-destructive and minimally-invasive technique
- Working cost is minimum
- Not special skills are required
- Large dataset
- Can be applied in both the laboratory and field

Disadvantages of the Chlorophyll Fluorescence Technique

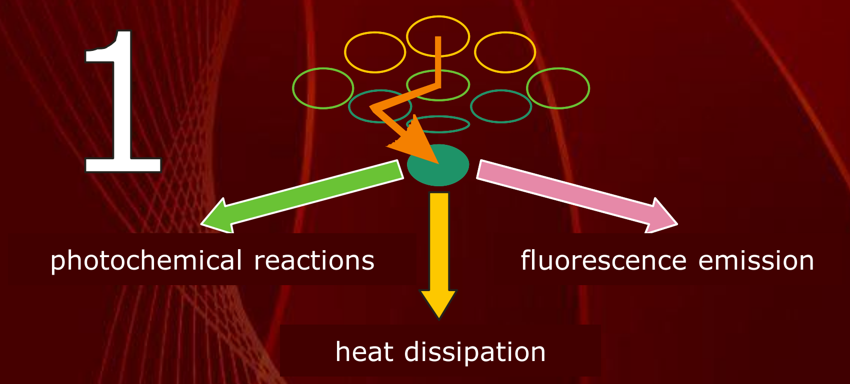
- Requires robust experimental planning
- Dataset interpretation is often complex
- Experimental and measurement errors are common and irreversible
- Equipment cost is high

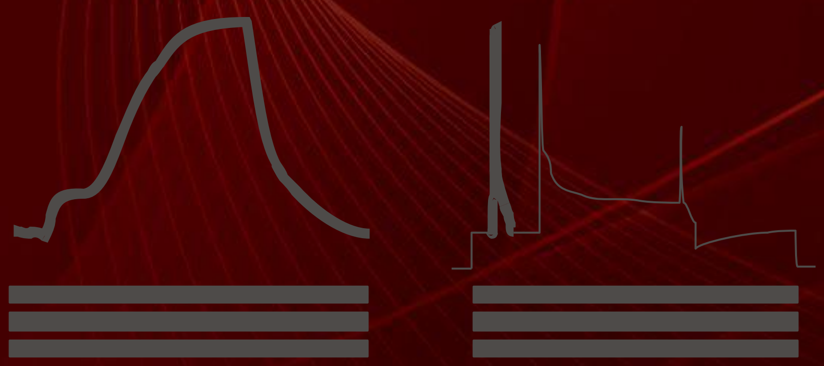
Explanation of Presentation



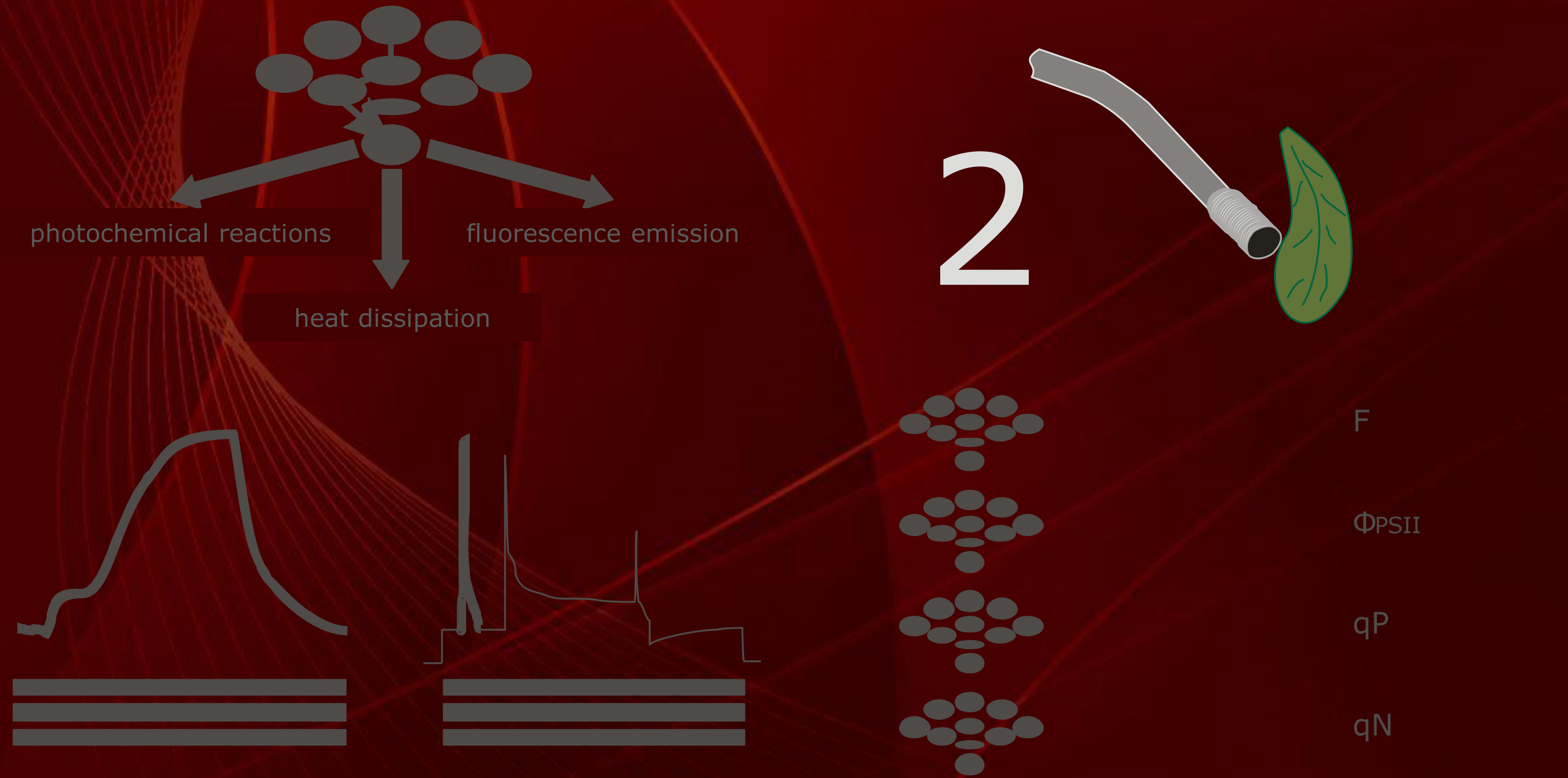
Visualization of the relative contribution of each energy quenching and relative changes in the intensity of emitted fluorescence depending on the type and intensity of radiation

1





Visualization of radiation (or radiations) applied on the sample



Visualization of radiation (or radiations) applied on the sample

Measuring radiation (ML): This is the only type of light whom the resulting fluorescence is recorded since it is the only fluorescence that is filtered electronically through the fluorometer. Usually red (650 nm) or blue (430 nm). PAR intensity is very low: < 0.15 μmol quanta m^{-2} s^{-1}).

Ακτινοβ.
μέτρησης
ML

Visualization of radiation (or radiations) applied on the sample

Saturation pulse (SP): This is the type of light with very high intensity that is applied for a very short time (up to 1 s) in order to fully saturate (fully reduce/close) all PSIIs. Usually red (650 nm), blue (430 nm) or white. PAR intensity is very high: up to 18000 μmol quanta m^{-2} s^{-1}.

Radiation Types Used in Chlorophyll Fluorescence

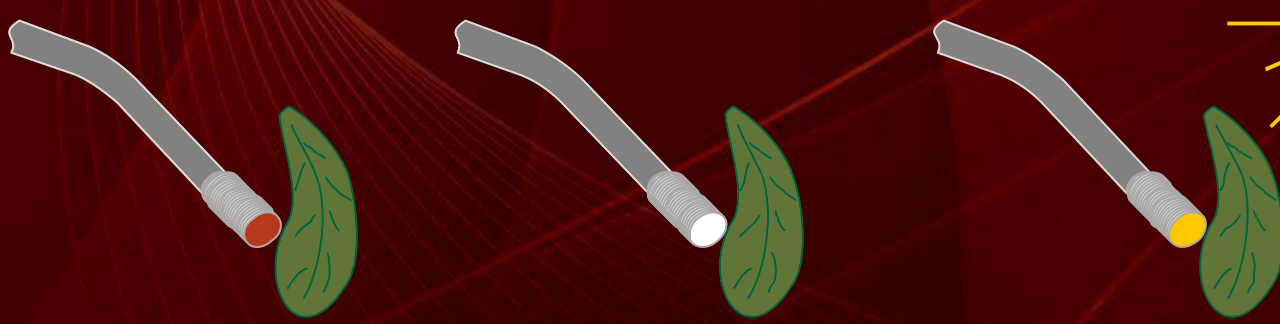
Visualization of radiation (or radiations) applied on the sample

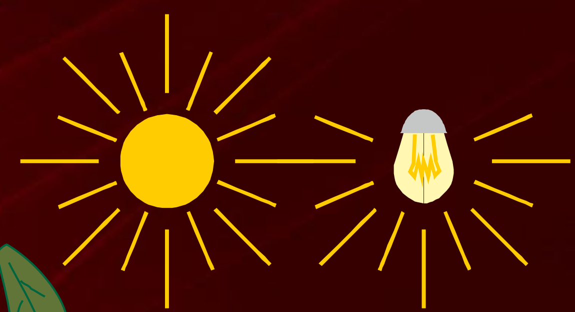
Actinic Light (AL): This is the photosynthetically /photochemically active (due to intensity and duration of illumination) that is either applied artificially or naturally existing. Usually red (650 nm), white (380-710 nm) or any other spectral composition within the PAR region. PAR intensity is variable (0-2000 μmol quanta m^{-2} s^{-1} or much higher).

Ακτινοβ. μέτρησης ML

Παλμός κορεσμού SP

Ακτινικό φως AL





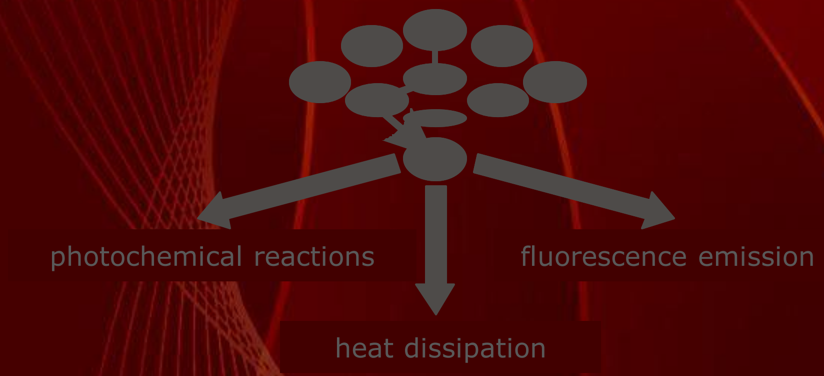
Radiation Types Used in Chlorophyll Fluorescence

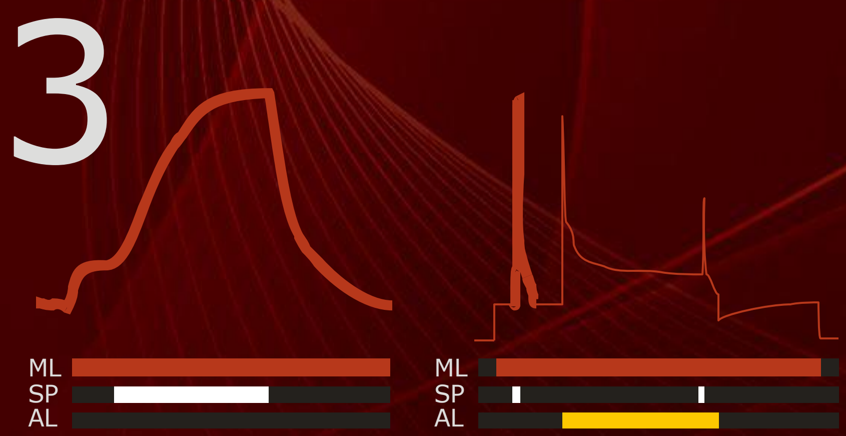
Visualization of radiation (or radiations) applied on the sample

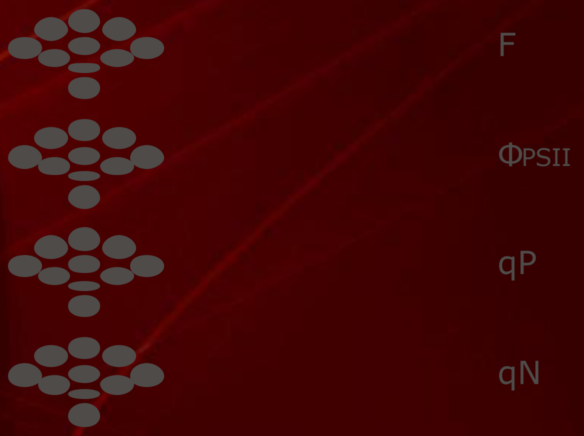
Far Red Light (FR): This is a special type of light that primarily excites PSI. Is used for the quick and efficient de-excitation of the electron transport chain Spectral composition is 730 nm, intensity is around 15 W m^{-2}.

Ακτινοβ. μέτρησης ML	Παλμός κορεσμού SP	Ακτινικό φως AL	Σκοτεινό ερυθρό FR

Fast and slow kinetic curves of chlorophyll fluorescence induction.

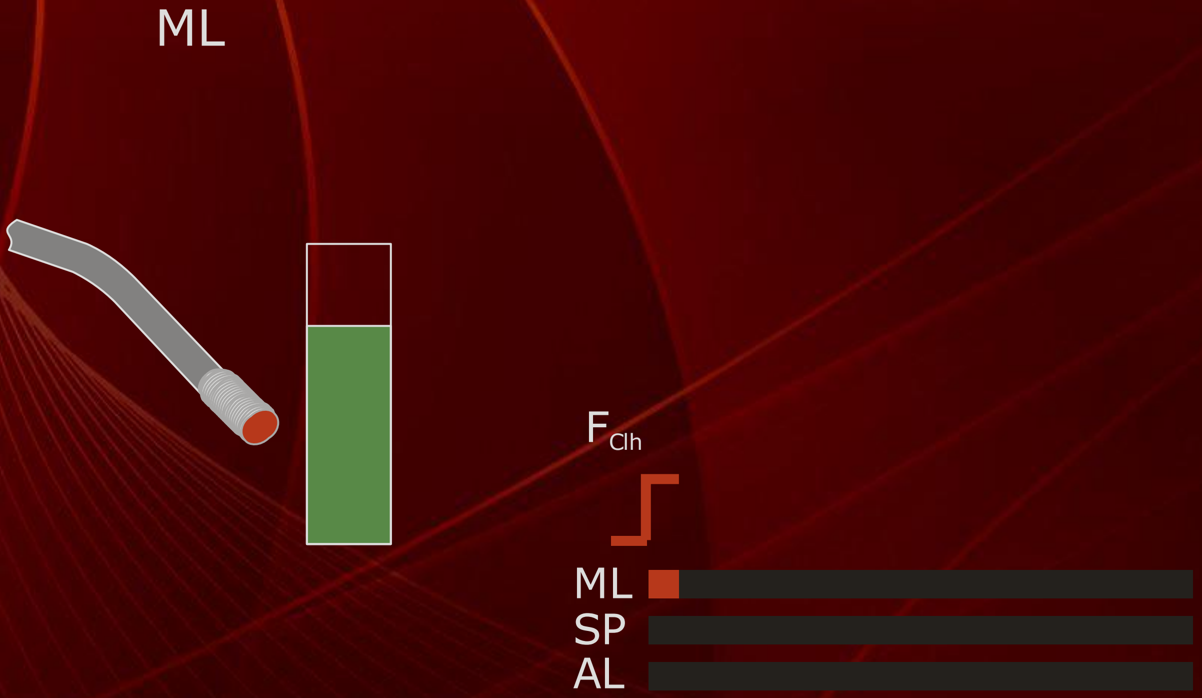






Measuring Radiation in PAM Fluorometry is Revolutionary

Fluorescence due to measuring radiation is separated opto-electronically from background fluorescence thus allowing measurements under any lighting regime



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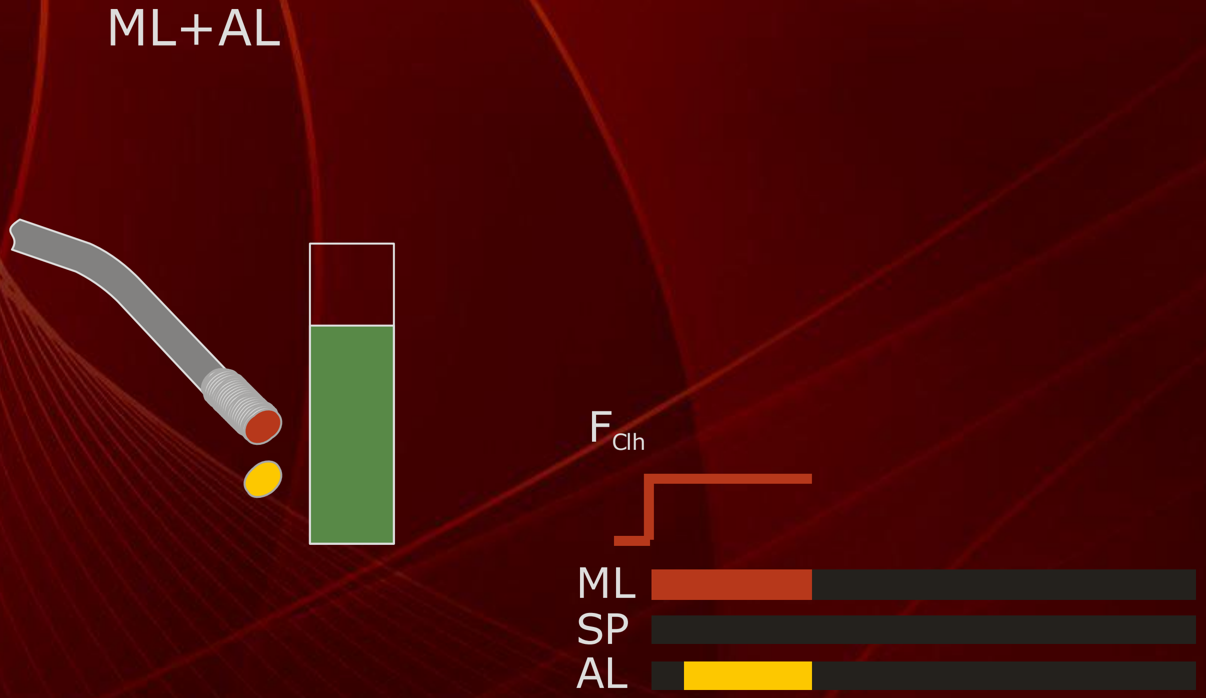

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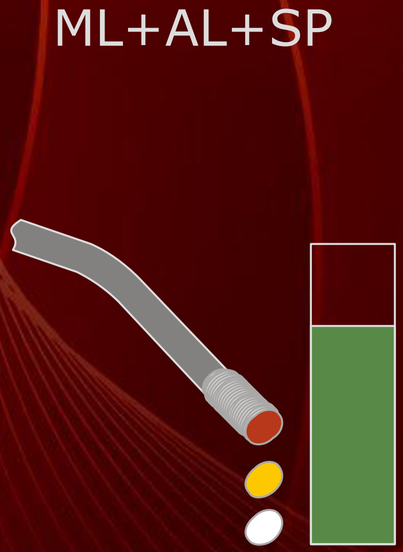

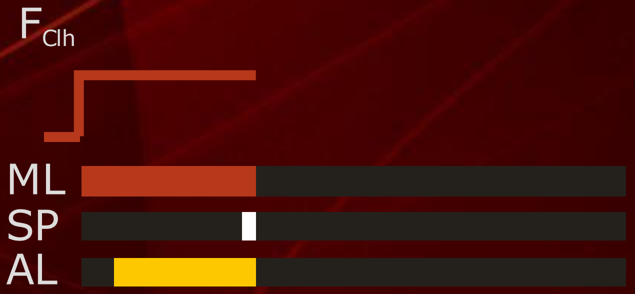

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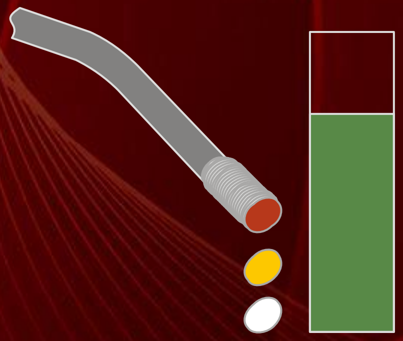
ML+AL+SP-ML



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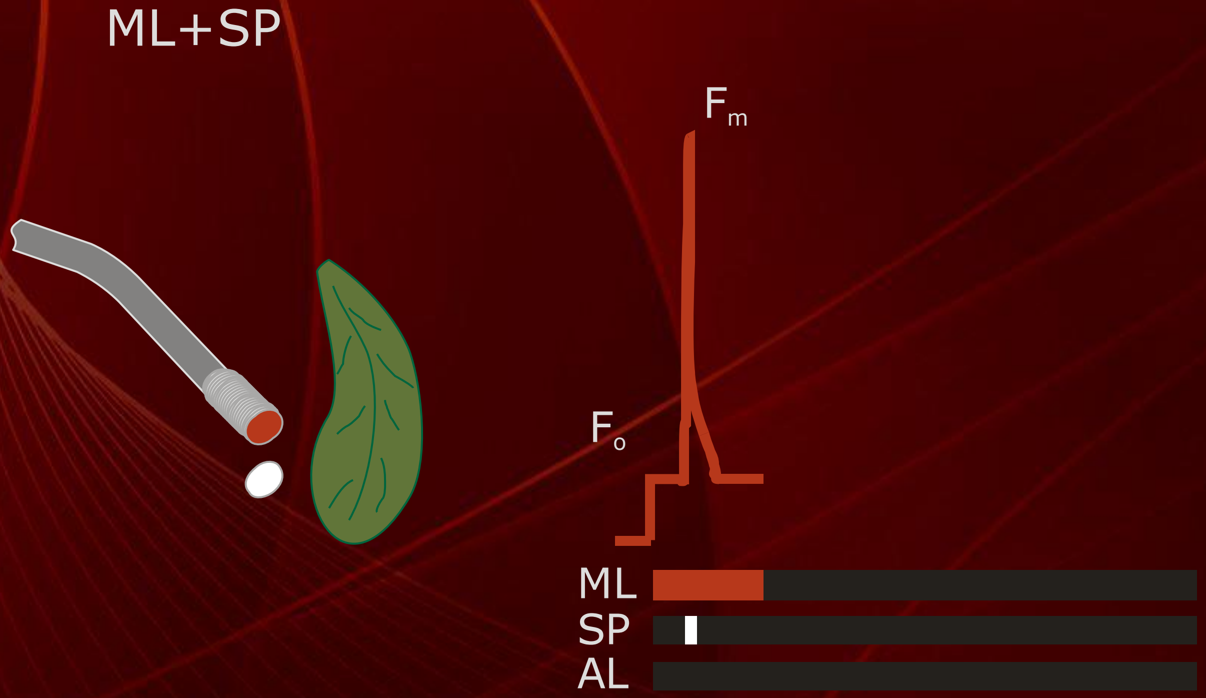

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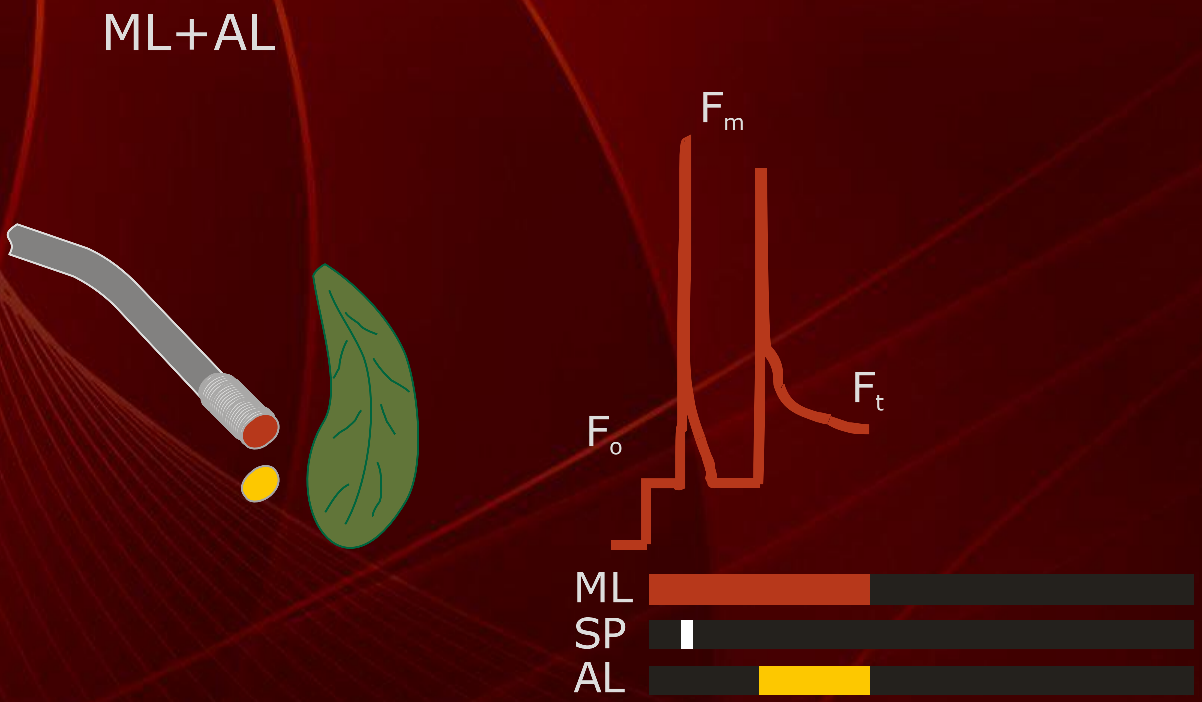

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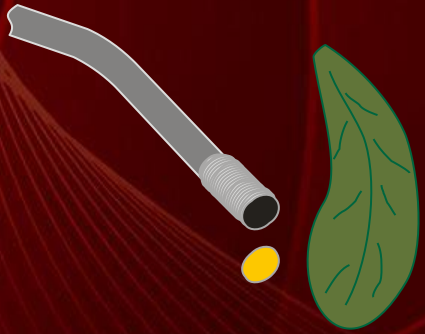

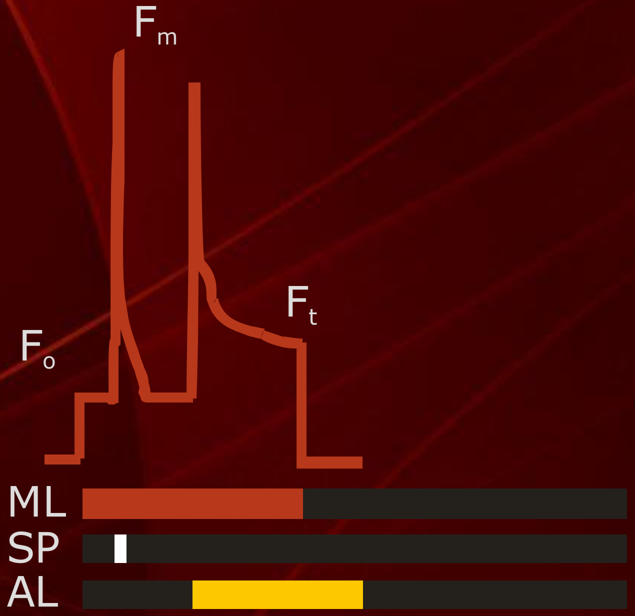

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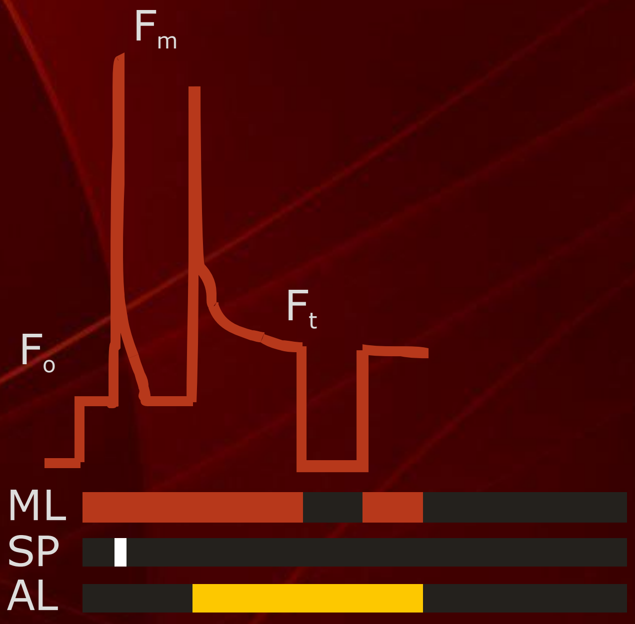

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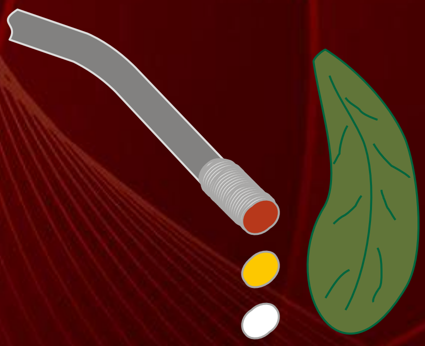

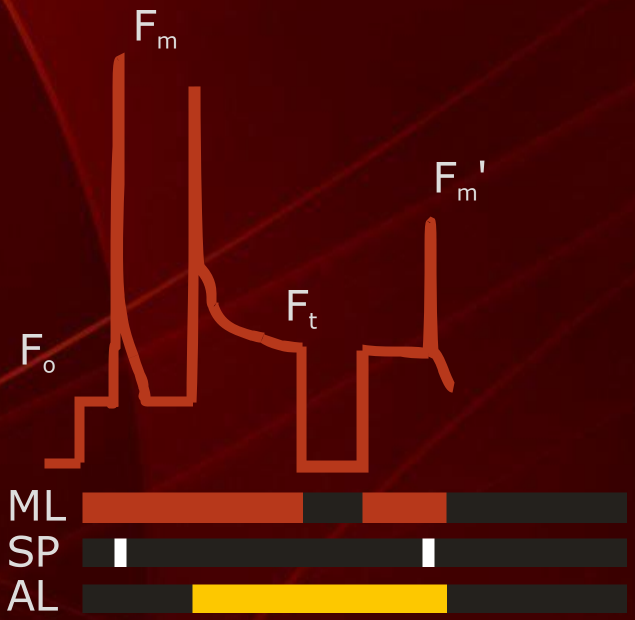

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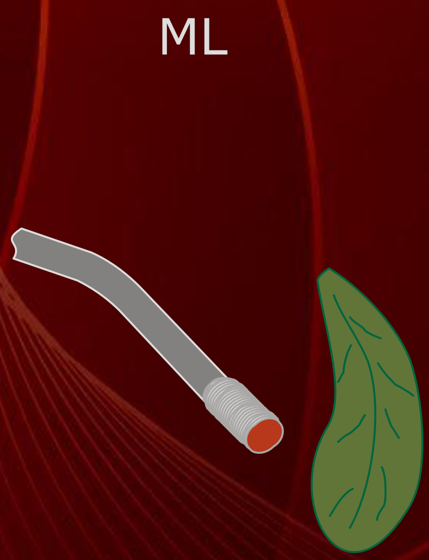

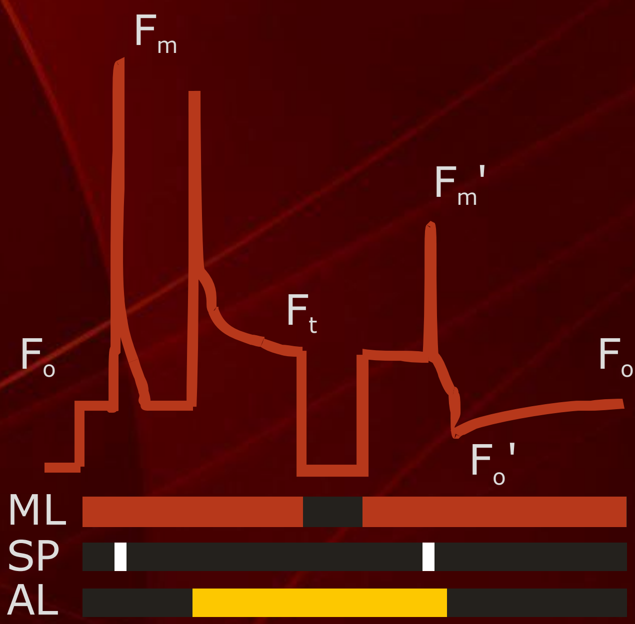

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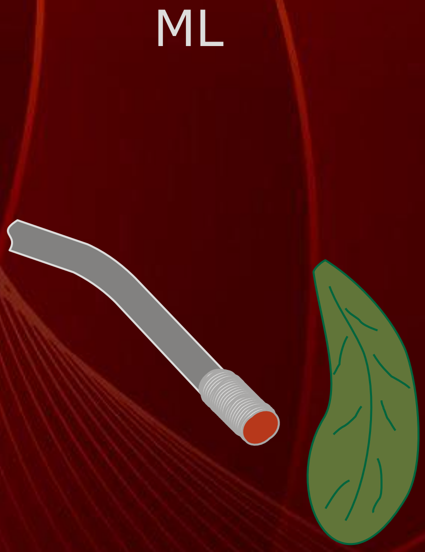
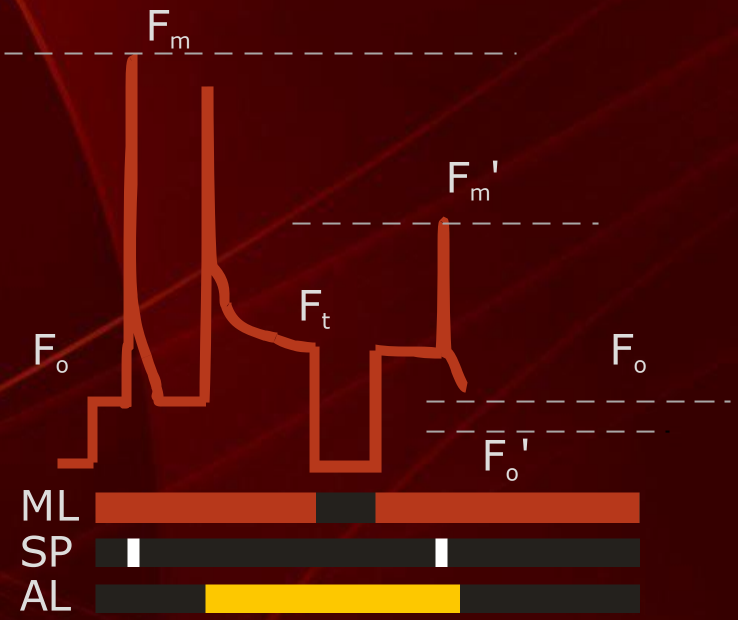
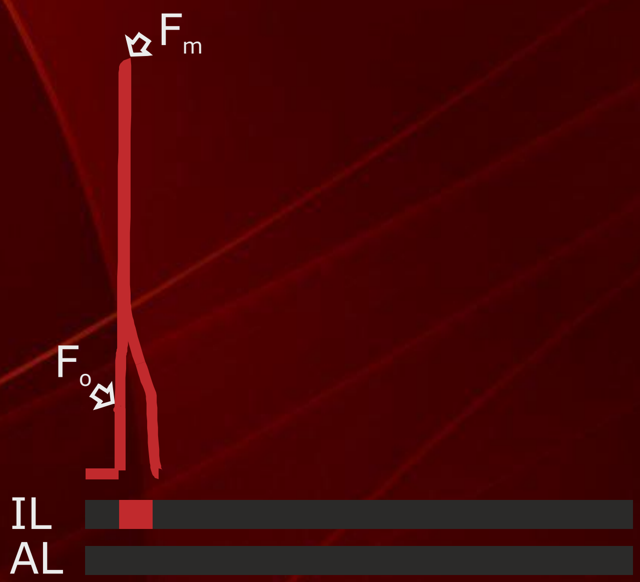




Early chlorophyll fluorometers were of stable amplitude modulated fluorescence and could only measure fluorescence yield in dark adapted samples (measuring only the maximum photochemical efficiency of PSII)

Induction Light (650 nm)
Μέγιστη ένταση 3000
μmol quanta m^{-2} s^{-1}



Evolution of Chlorophyll Fluorescence Instrumentation

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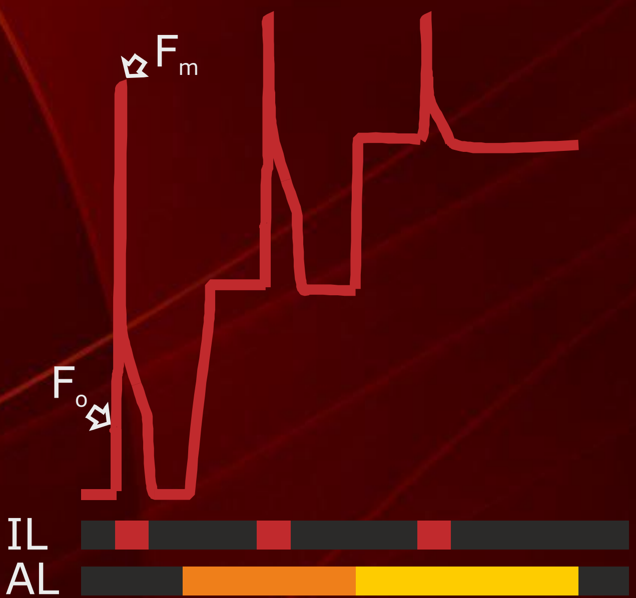

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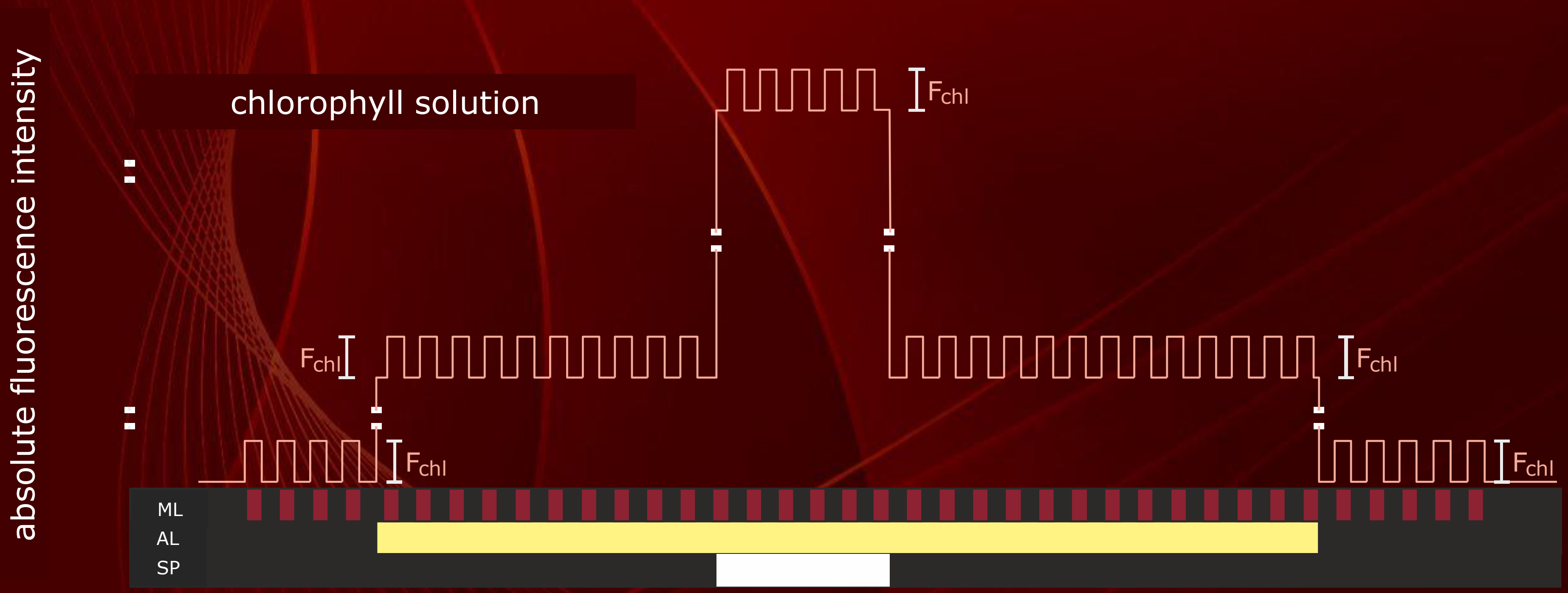

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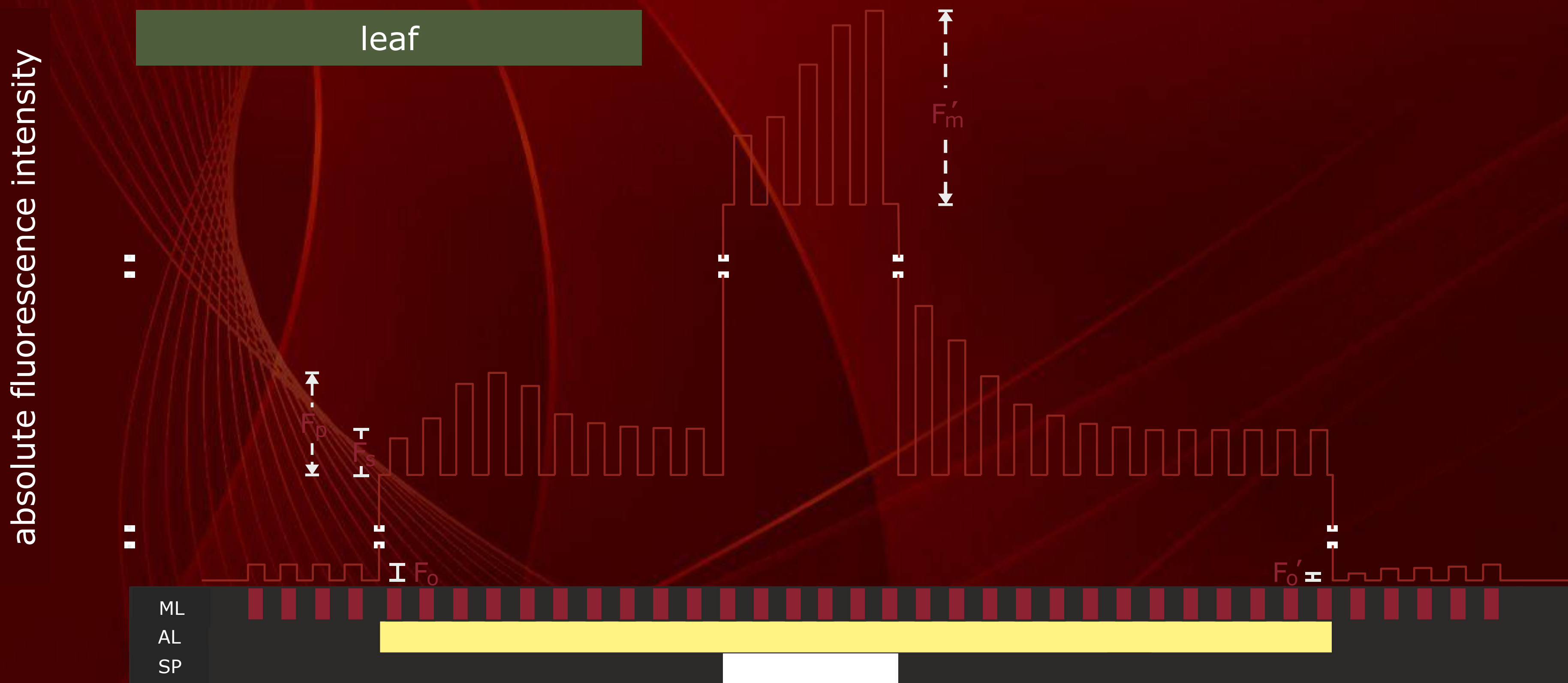

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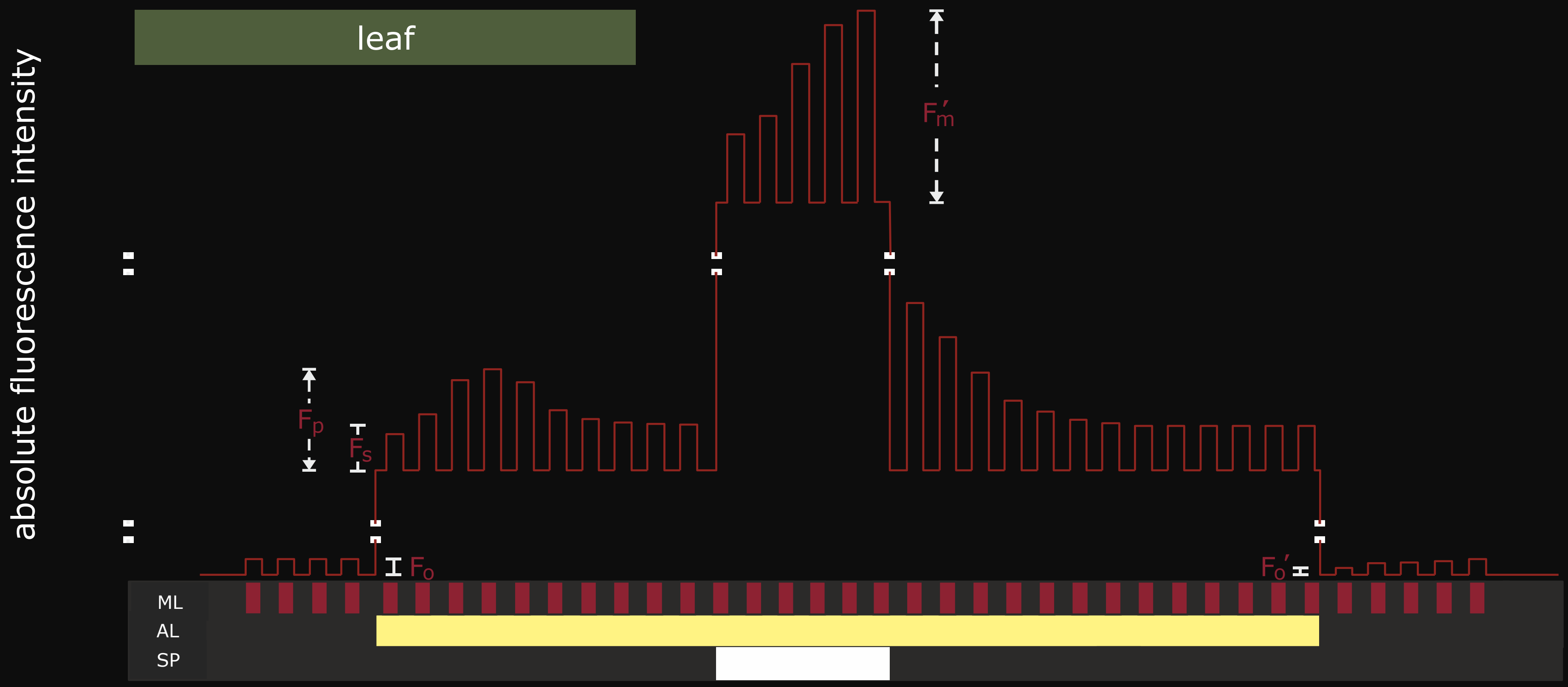

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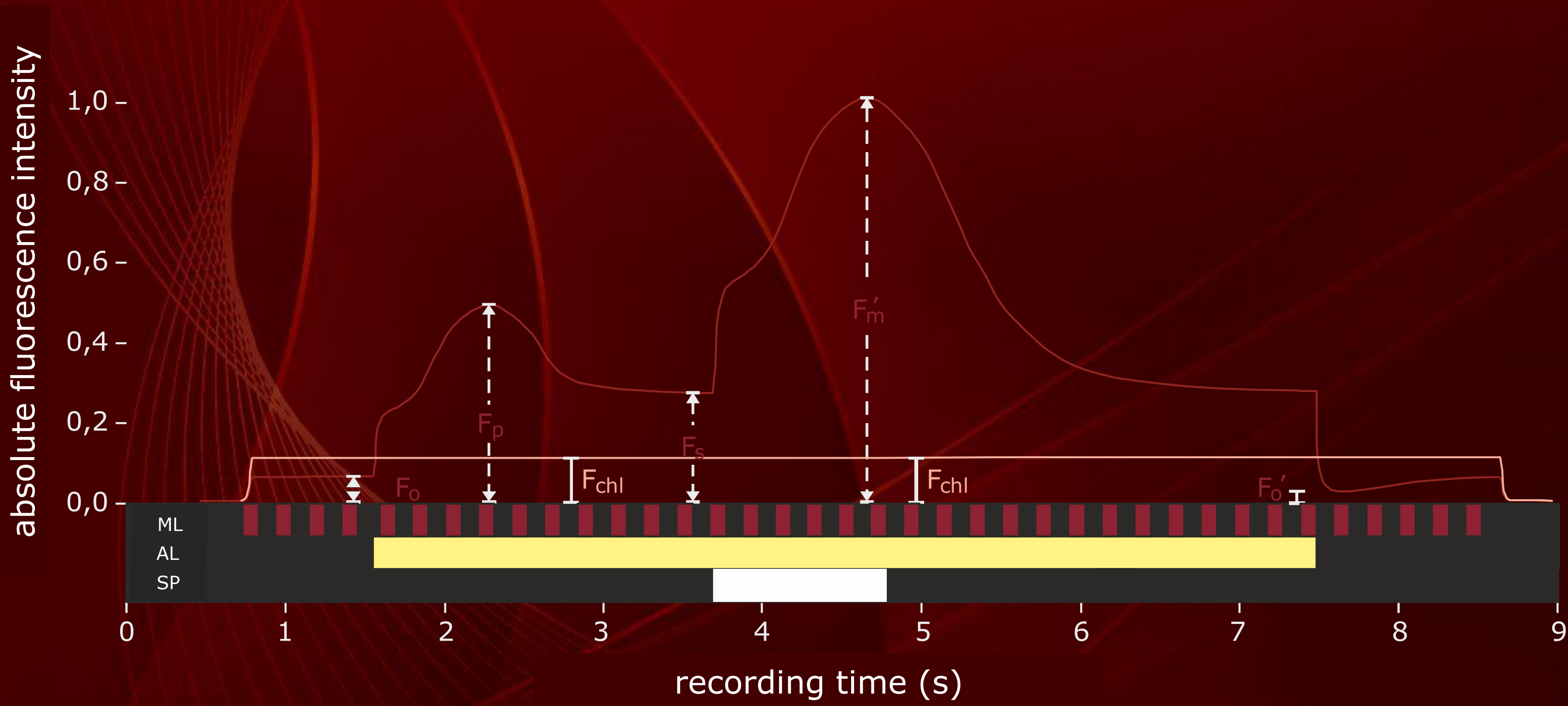
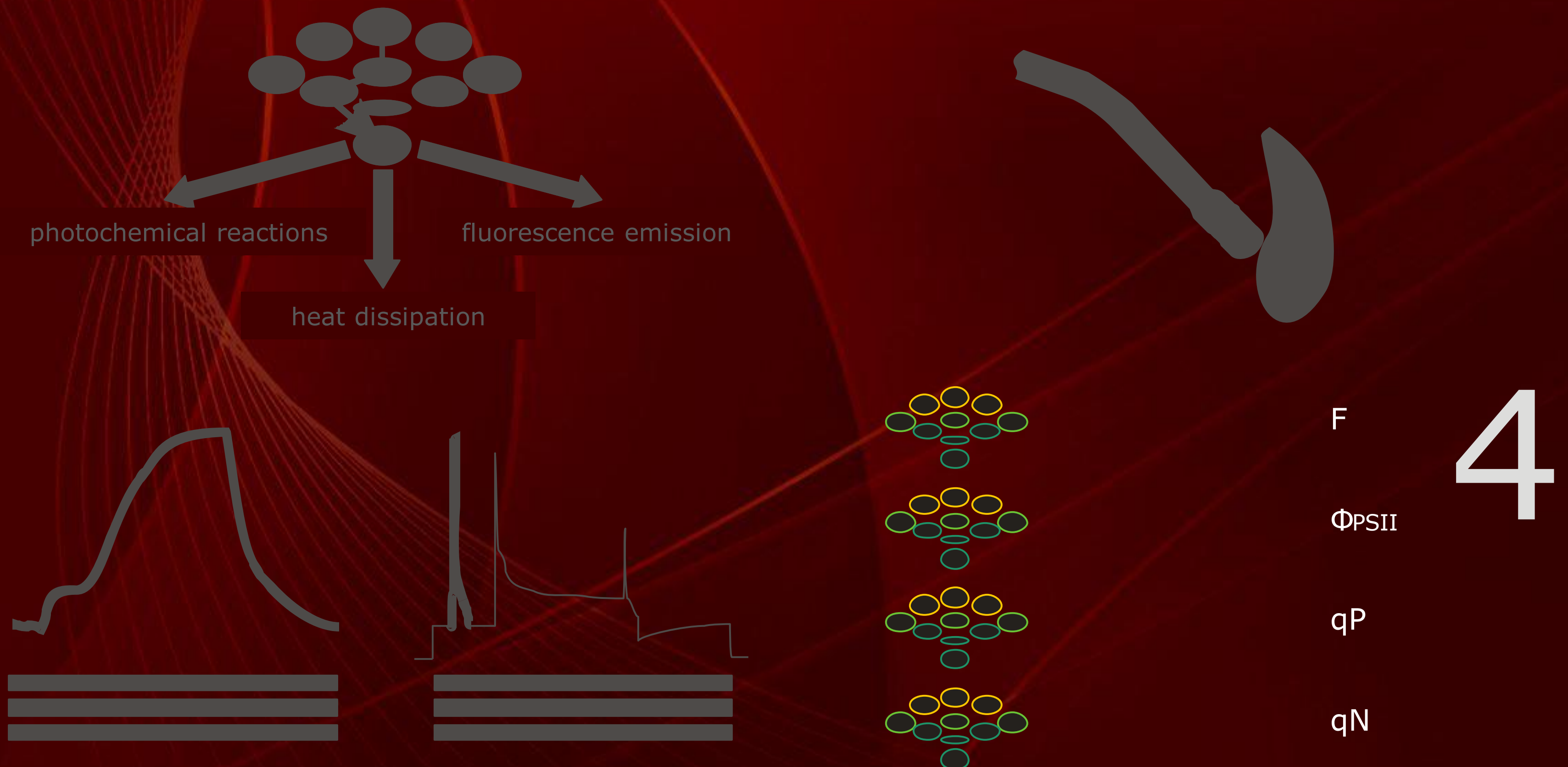


Visualization of energy state of the population of PS II and indicative values of photochemical parameters



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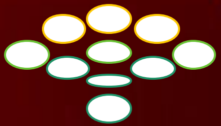
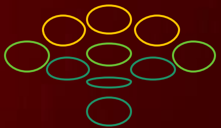
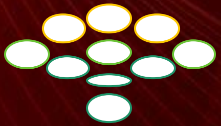


F 325



Φ_{PSII} 0,248

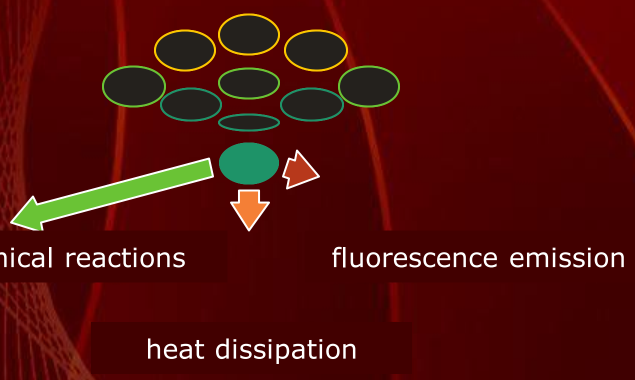
qP 0,507

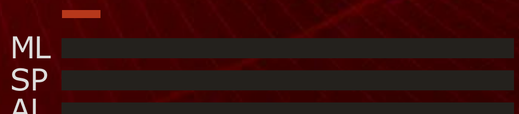


qN 0,872

Analysis of Fast Kinetics Fluorescence Induction Curve

In a 'dark adapted' leaf, all PSIIs are oxidized. Photochemical quenching is 'potentially' maximal while non-photochemical processes are inactive.





F_0

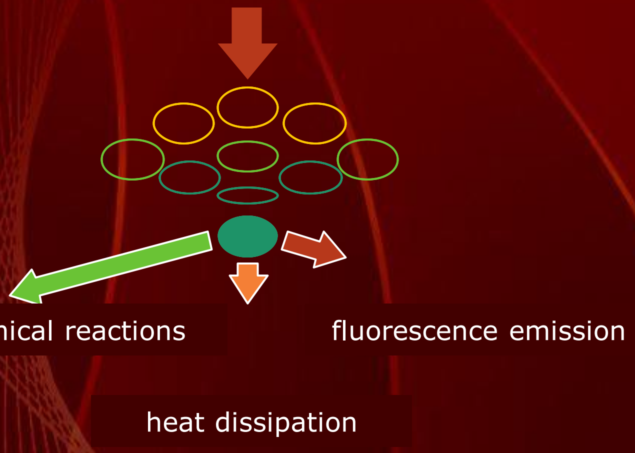
Φ_{PSII} maximal

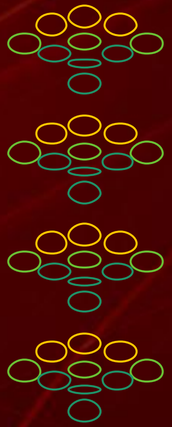
qP maximal (1)

qN minimal (0)

Analysis of Fast Kinetics Fluorescence Induction Curve

In this sample, application of measuring light will result is recording of the 'basic fluorescence yield', F_o





F F_o

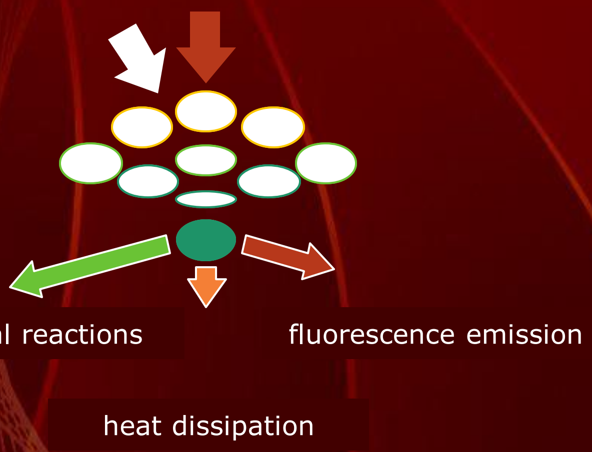
Φ_{PSII} maximal

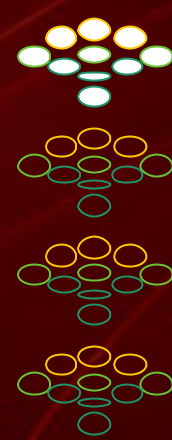
qP maximal (1)

qN minimal (0)

Analysis of Fast Kinetics Fluorescence Induction Curve

Application of a saturation pulse will start closing photosystems resulting in a transient of the fluorescence level up to a plateau which appears constant while reduction of photosystems and primary electron acceptors is completed

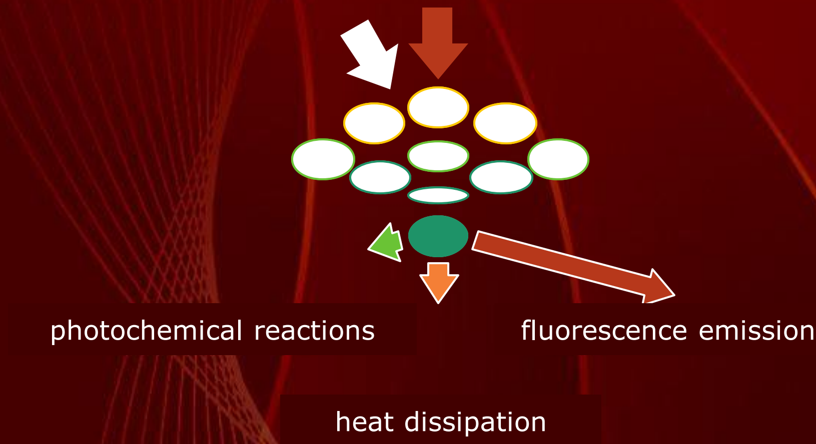


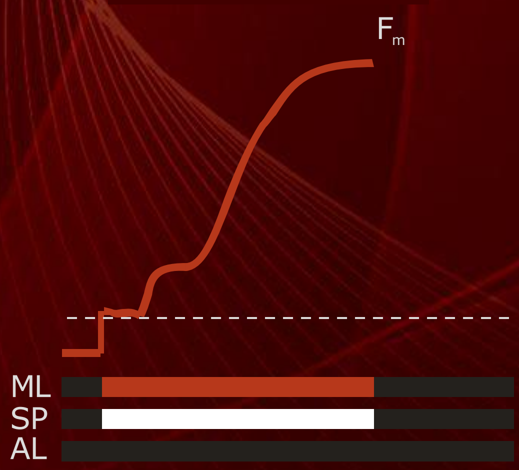


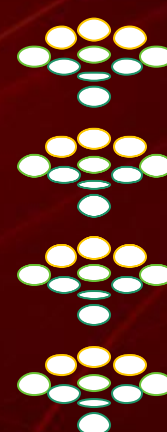
F	F_i
Φ_{PSII}	-----
qP	<1
qN	0

Analysis of Fast Kinetics Fluorescence Induction Curve

Quite suddenly the continued application of the saturation pulse closes all photosystems resulting in an abrupt rise of fluorescence in a maximal level which is referred as the 'maximum fluorescence', F_m



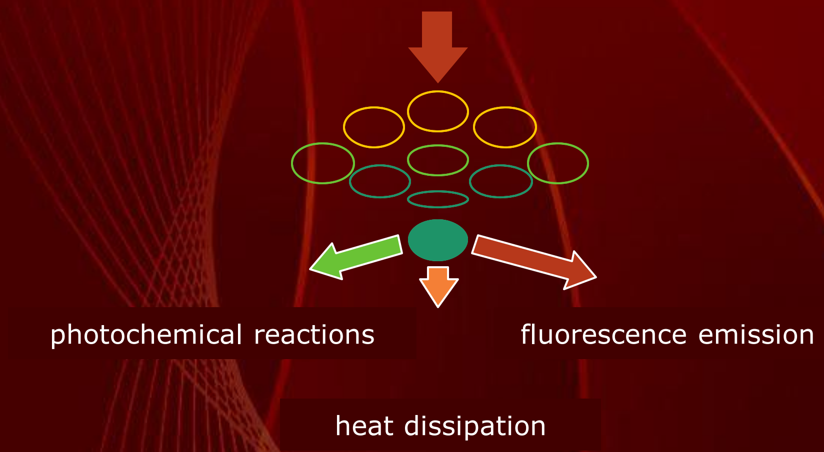


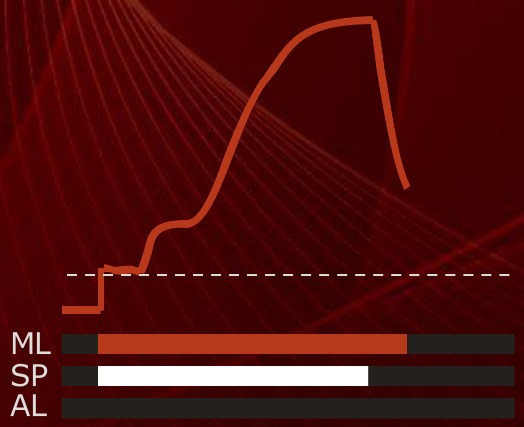


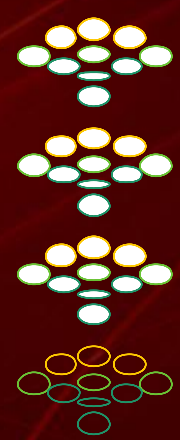
F	F_m
Φ_{PSII}	0.825
qP	0
qN	0

Analysis of Fast Kinetics Fluorescence Induction Curve

Ending of the saturation pulse results in a abrupt drop of the fluorescence due to photochemical quenching of energy as the electron transport chain begins to operate



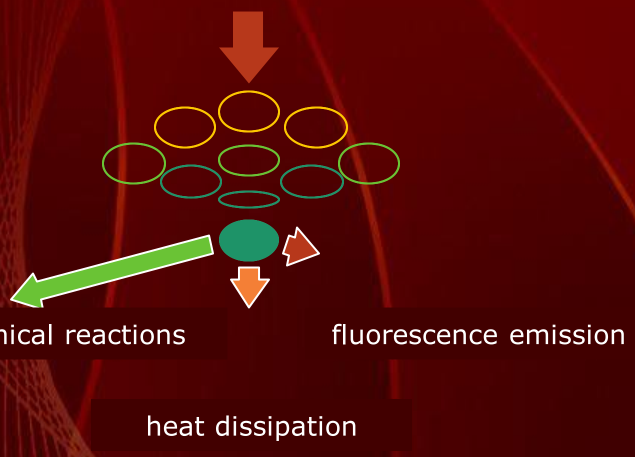


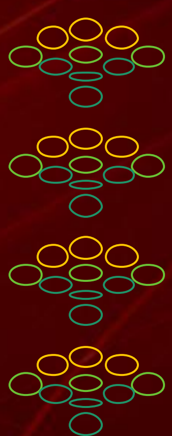


F	$<F_m$
Φ_{PSII}	0.825
qP	>0
qN	0

Analysis of Fast Kinetics Fluorescence Induction Curve

At the end, fluorescence emission returns to basic levels of F_o since all photosystems have been gradually fully oxidized

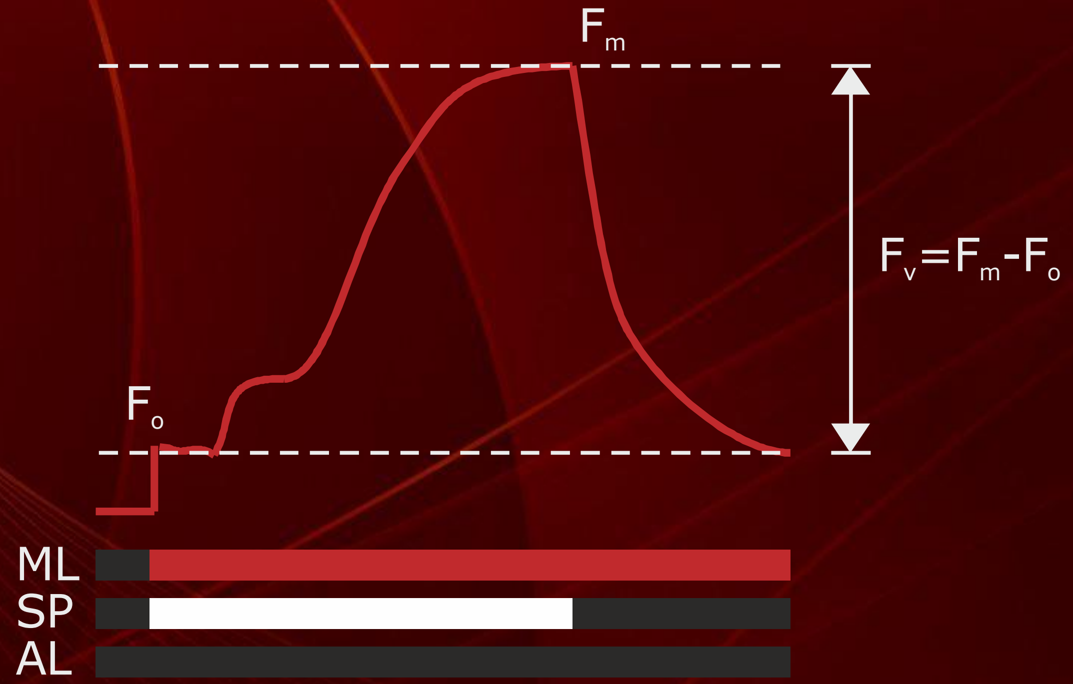




F	F_o
Φ_{PSII}	0.825
qP	1
qN	0

Analysis of Fast Kinetics Fluorescence Induction Curve

Maximum quantum efficiency of PS II ($\Phi_{PSIIo}=(F_m-F_o)/F_m=F_v/F_m$ is a measure of the ability of PS II to absorb light energy and driving photochemical electron flow



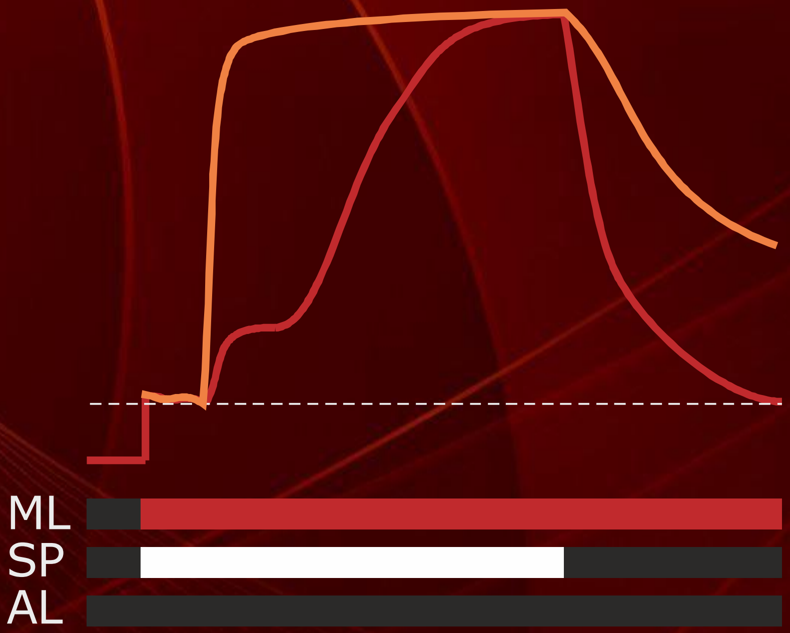
Analysis of Fast Kinetics Fluorescence Induction Curve

Φ_{PSIIo} represents the most common index of damage due to stressors related to photosynthesis and membrane intactness as well as of the various biochemical components of chloroplasts. Stressors include photooxidation, nutrient deficiencies, toxicities, salinity, frost, etc.

parameter	healthy plant	stressed plant
F_o	508	703
F_m	2904	2003
F_v	2396	1300
Φ_{PSIIo}	0.825	0.649

Analysis of Fast Kinetics Fluorescence Induction Curve

Which would be the fluorescence induction curve of a sample in which DCMU (an inhibitor of electron transport chain) had been applied?



Parameters of Fast Kinetics Fluorescence Induction Curve

F_o, Basic fluorescence. Related to the ability of energy flow from the light harvesting antenna to the PS II reaction center.

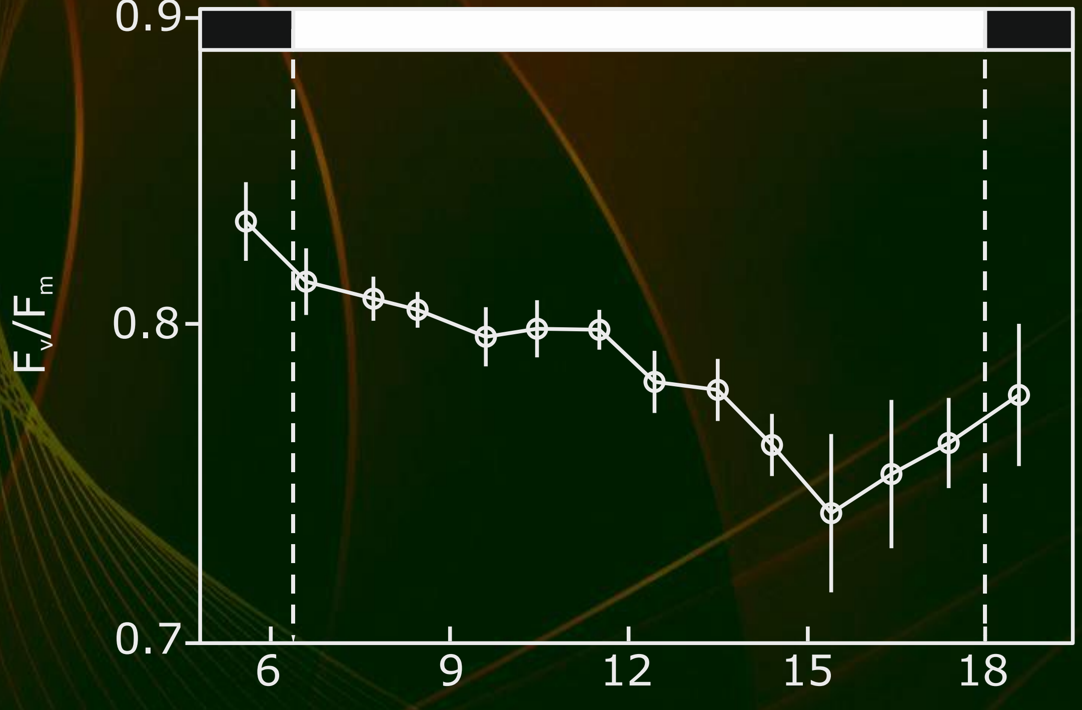
F_m, Maximum fluorescence. Is a measure of total energy flow capacity through the PS II.

F_v, Variable fluorescence. Related to the energy flow from the PSII reaction center to the electron transport chain (total energy minus energy loss recorded as F_o).

$F_v/F_m=(F_m-F_o)/F_m=\Phi_{PSIIo}$, Maximum photochemical efficiency of PSII. Related to the maximum percent of energy flow towards the electron transport chain related to the total energy absorbed in PS II.

Practical Issues Concerning the Fast Kinetics Induction Curve

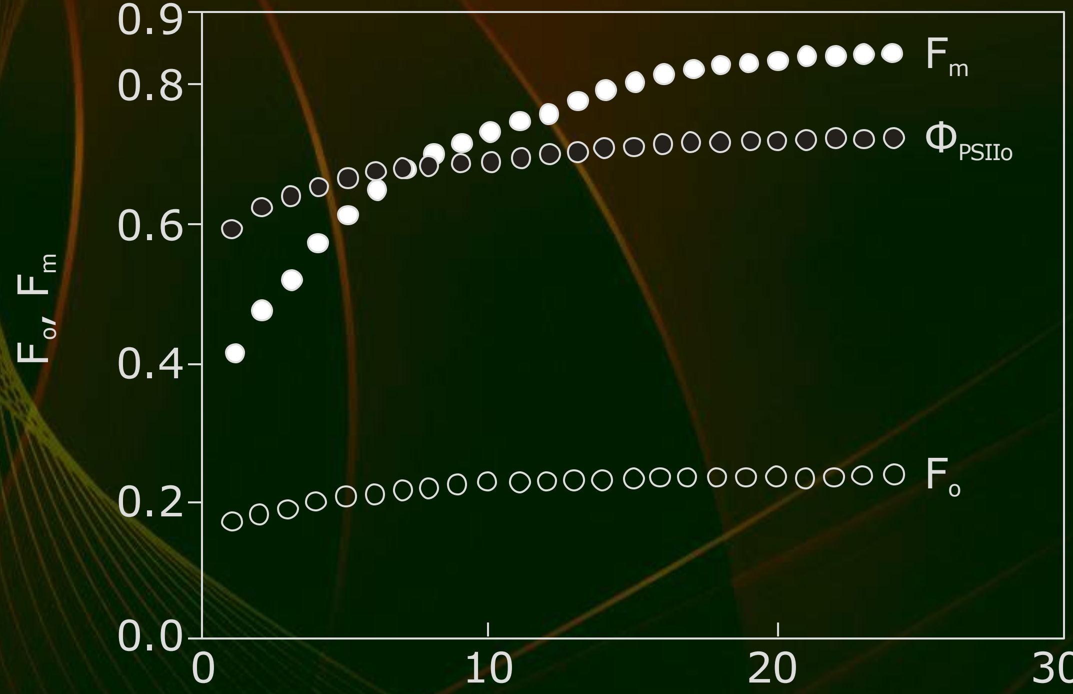
When during the 24h day/night period should we measure the Φ_{PSIIo} parameter?



time of day

Practical Issues Concerning the Fast Kinetics Induction Curve

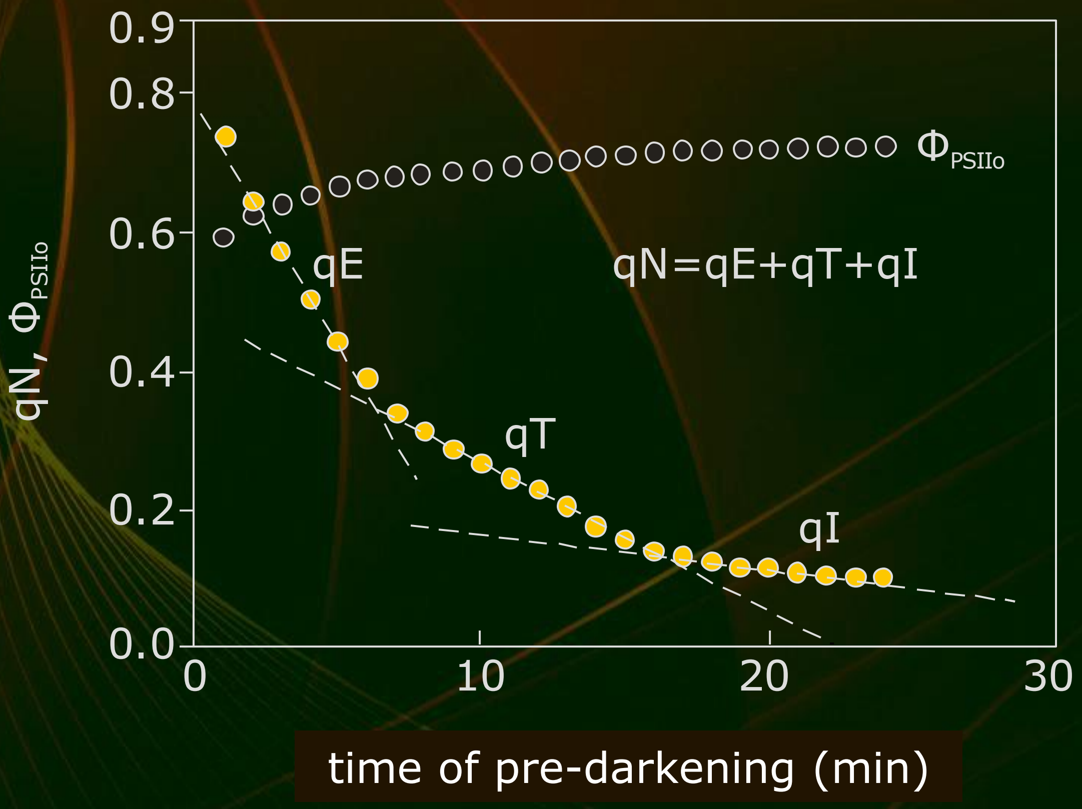
Which is the minimum darkening time to achieve a reliable measurement of the Φ_{PSIIo} parameter?



time of pre-darkening (min)

Practical Issues Concerning the Fast Kinetics Induction Curve

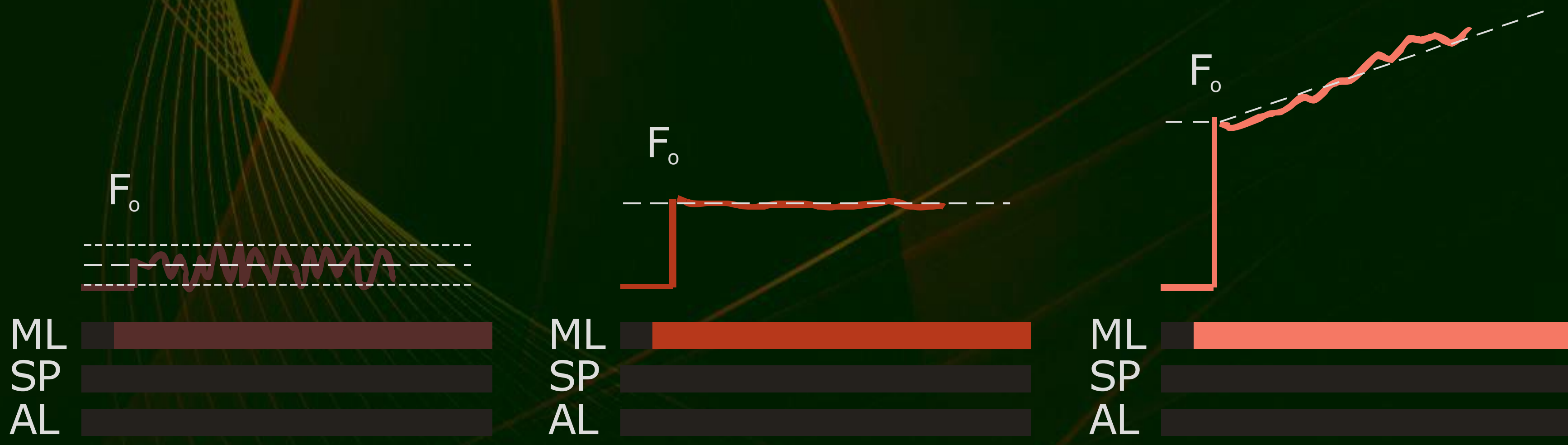
Which is the minimum darkening time to achieve a reliable measurement of the Φ_{PSIIo} parameter?



Practical Issues Concerning the Fast Kinetics Induction Curve

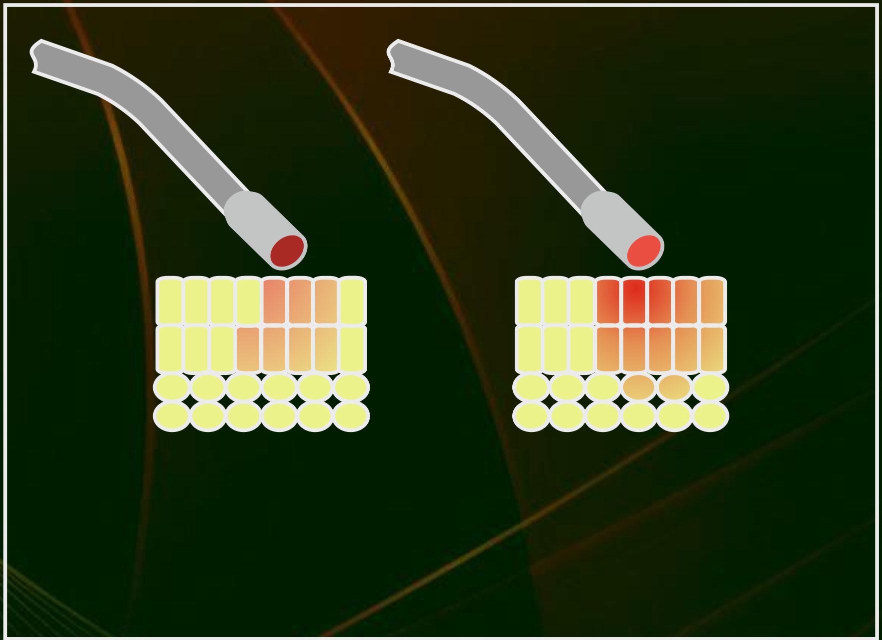
How do we choose the right measuring light intensity?

very low ML intensity	optimal ML intensity	very high ML intensity



Practical Issues Concerning the Fast Kinetics Induction Curve

Since ML intensity is so important could we resetting it during an experimental dataset acquisition?



Practical Issues Concerning the Fast Kinetics Induction Curve

Is it proper to compare between samples or treatments using the absolute fluorescence yield values (i.e. F_o,F_v and F_m)?

ML intensity	F_o	F_m	Φ_{PSIIo}
6	304	1624	0.813
9	458	2430	0.812

Chl concentration	F_o	F_m	Φ_{PSIIo}
1.0x	294	1407	0.791
1.2x	317	1655	0.808

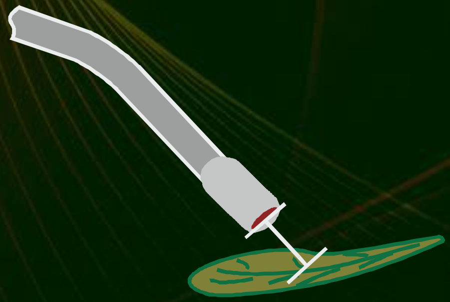
Practical Issues Concerning the Fast Kinetics Induction Curve

Is it proper to compare between samples or treatments using the absolute fluorescence yield values (i.e. F_o,F_v and F_m)?

Factors influencing absolute fluorescence yield values:

Geometry of the optical apparatus

1) Distance between the exit plane of the optical fiber and the sample



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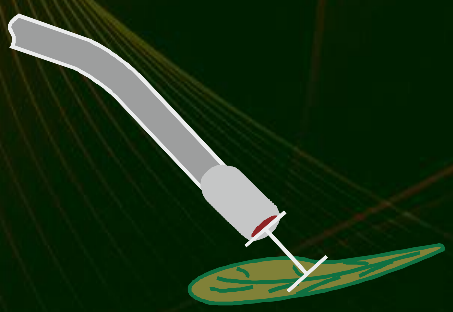
1) Distance between the exit plane of the optical fiber and the sample

2) Angle between light direction and leaf plane



Practical Issues Concerning the Fast Kinetics Induction Curve

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Factors influencing absolute fluorescence yield values:
Fluorometer operational parameters
3) Measuring light intensity (e.g. ML between 1 and 12)

Practical Issues Concerning the Fast Kinetics Induction Curve

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5) ML Burst operation (ML radiation lasts 0.2 s per 1 s period)

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6) Electronic Gain (e.g. between 1 to 12)

Practical Issues Concerning the Fast Kinetics Induction Curve

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4) Measuring light pulse frequency (e.g. 600 Hz and 20 kHz)
5) ML Burst operation (ML radiation lasts 0.2 s per 1 s period)
6) Electronic Gain (e.g. between 1 to 12)
7) Intactness of the optical fiber and the halogen lamp

Practical Issues Concerning the Fast Kinetics Induction Curve

Is it proper to compare between samples or treatments using the absolute fluorescence yield values (i.e. F_o,F_v and F_m)?

Factors influencing absolute fluorescence yield values:
Anatomical and Physiological parameters of the sample
8) Structure and morphology of the sample surface

Practical Issues Concerning the Fast Kinetics Induction Curve

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8) Structure and morphology of the sample surface
9) Presence of trichome or pigments (e.g. anthocyanins)

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Anatomical and Physiological parameters of the sample

8) Structure and morphology of the sample surface

9) Presence of trichome or pigments (e.g. anthocyanins)

10) Sample thickness, presence of sclerenchyma, cell wall thickness

Practical Issues Concerning the Fast Kinetics Induction Curve

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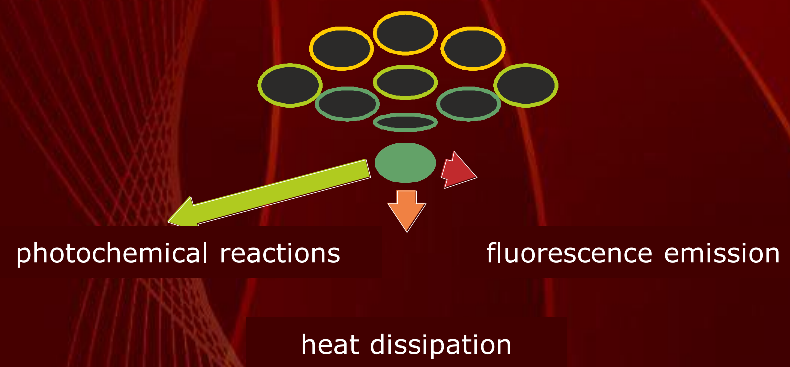
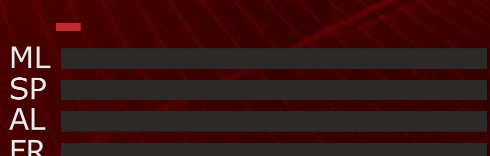
Anatomical and Physiological parameters of the sample
8) Structure and morphology of the sample surface
9) Presence of trichome or pigments (e.g. anthocyanins)
10) Sample thickness, presence of sclerenchyma, cell wall thickness
11) Concentration of chlorophylls

Analysis of Slow Kinetics Fluorescence Induction Curve

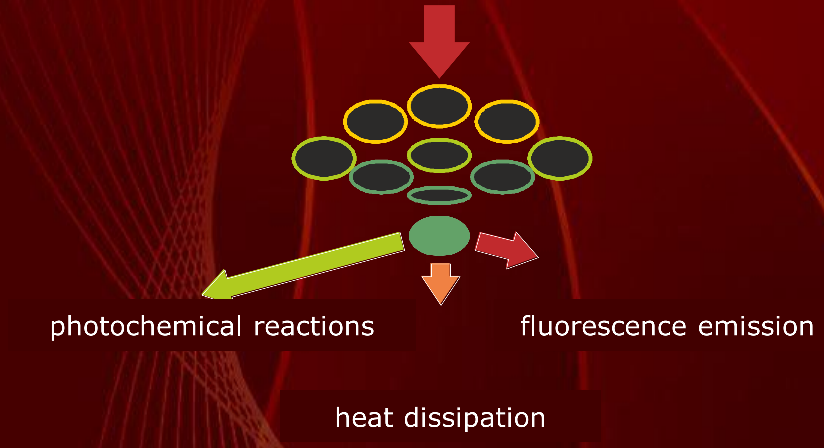
In a 'dark adapted' leaf, all PSIIs are oxidized. Photochemical quenching is 'potentially' maximal while non-photochemical processes are inactive.

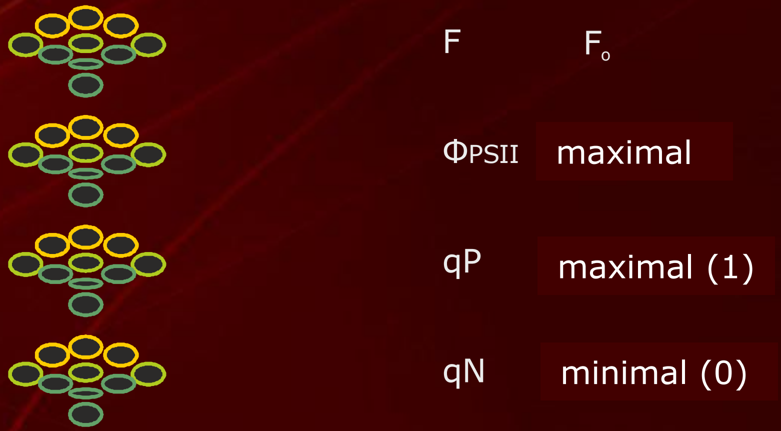




Analysis of Slow Kinetics Fluorescence Induction Curve

In this sample, application of measuring light will result is recording of the 'basic fluorescence yield', F_o

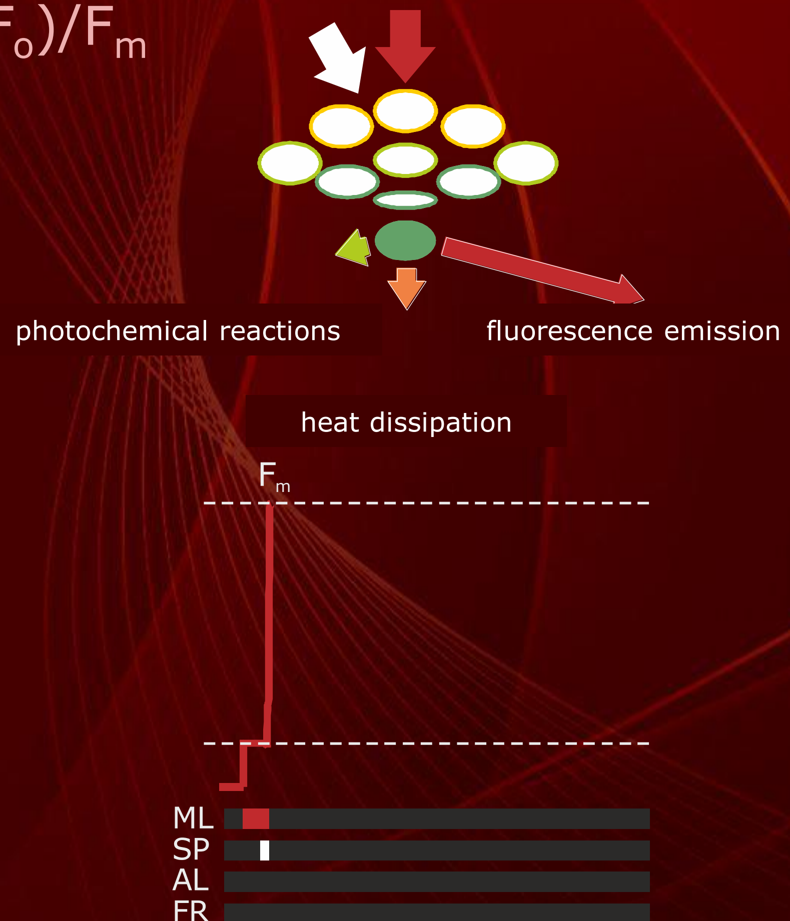


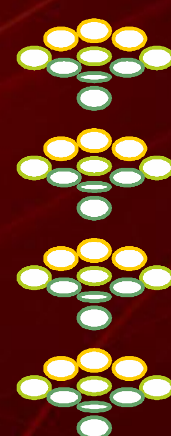


Analysis of Slow Kinetics Fluorescence Induction Curve

Application of a saturation pulse allows the recording of the maximum fluorescence yield 'in the dark', F_m

Through the F_o and F_m parameters we may calculate the maximum quantum yield of PS II (Φ_{PSIIo}) equal to $F_v/F_m=(F_m-F_o)/F_m$

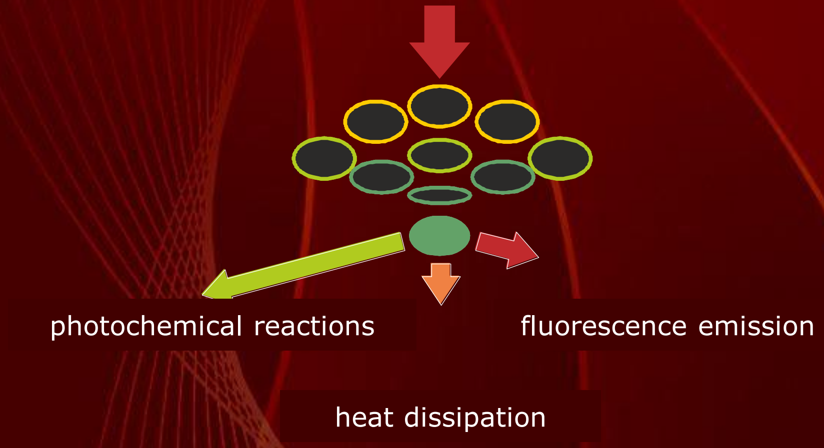


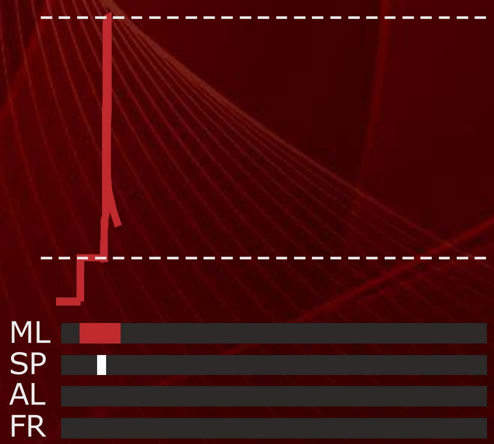


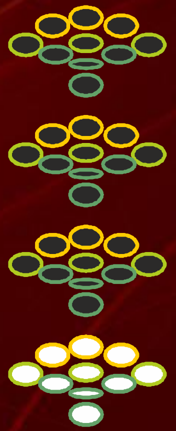
F	F_m
ΦPSII	0.825
qP	0
qN	0

Analysis of Slow Kinetics Fluorescence Induction Curve

Fluorescence decay back to F_o is fast since there is no actinic light present

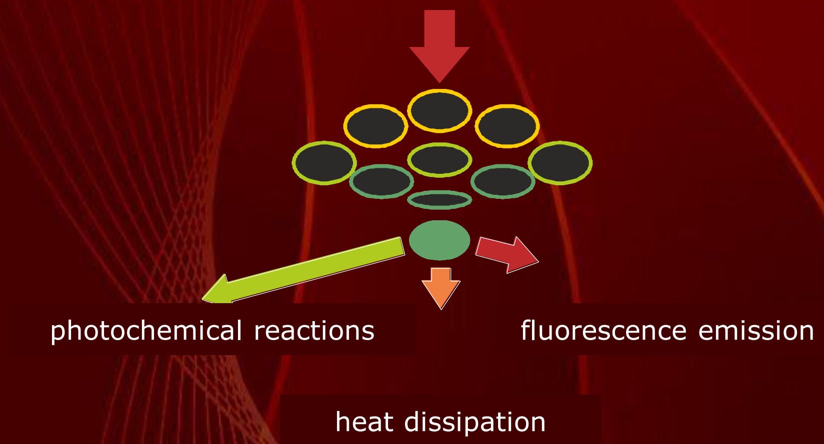


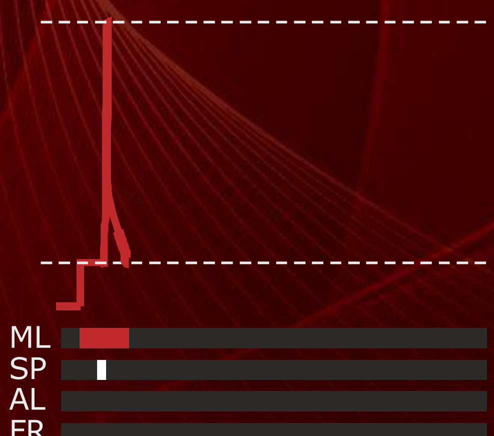


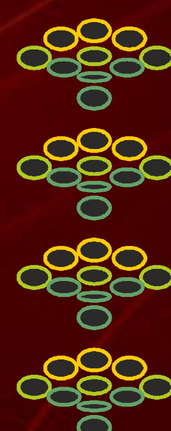


F	F_t
ΦPSII	0.825
qP	maximal (1)
qN	minimal (0)

Analysis of Slow Kinetics Fluorescence Induction Curve



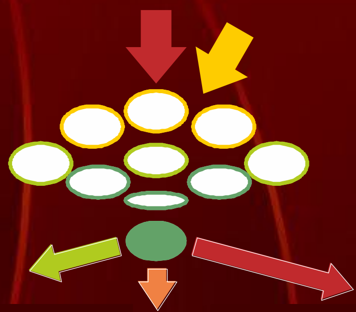


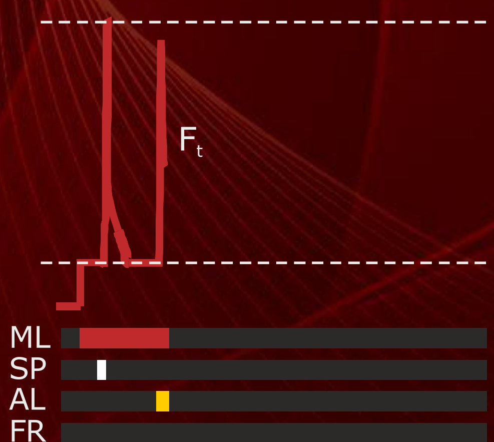


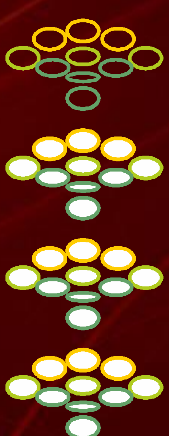
F	F_o
Φ_{PSII}	0.825
qP	maximal (1)
qN	minimal (0)

Analysis of Slow Kinetics Fluorescence Induction Curve

Switching on actinic light causes a transient of fluorescence which decays fast as electron transport chain begins to quench energy



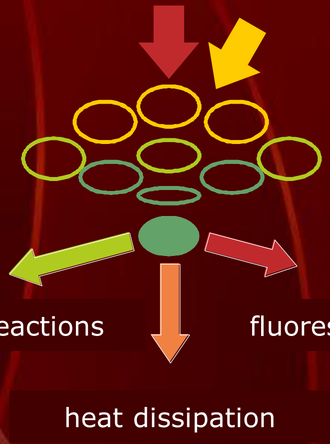


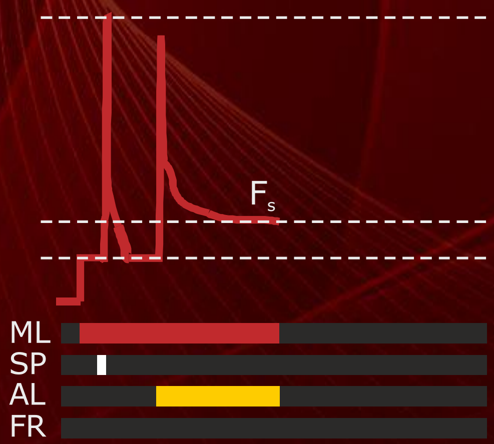


F	F_t
ΦPSII	0.825
qP	maximal (1)
qN	minimal (0)

Analysis of Slow Kinetics Fluorescence Induction Curve

However, basic level of fluorescence is not attained but, depending on actinic light levels, a level of fluorescence F_s, higher than F_o is observed since actinic light causes a relative reduction of reaction centers

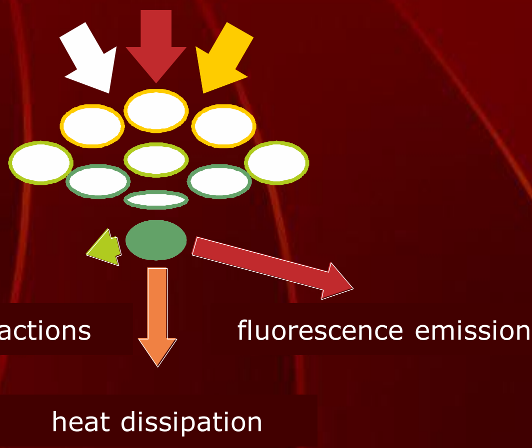


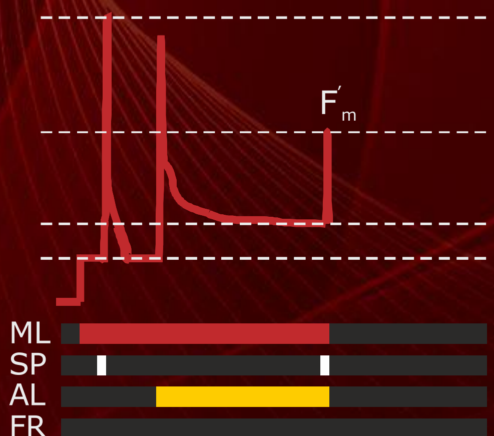


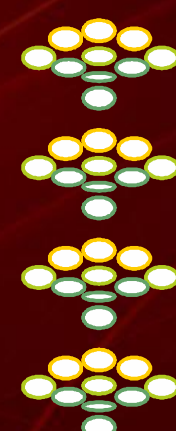
F	F_s
Φ_{PSII}	0.825
qP	<1
qN	>0

Analysis of Slow Kinetics Fluorescence Induction Curve

Under these conditions, the application of a saturation pulse causes a transient to a maximum fluorescence level ‘in the light’, the F_m' parameter. F_m' levels are always lower than F_m since some PSII reaction center reduction lowers the energy capacity.







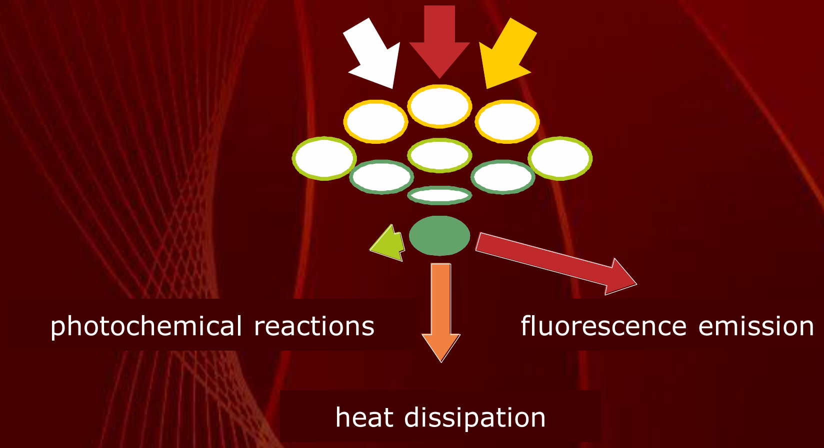
F F_m'

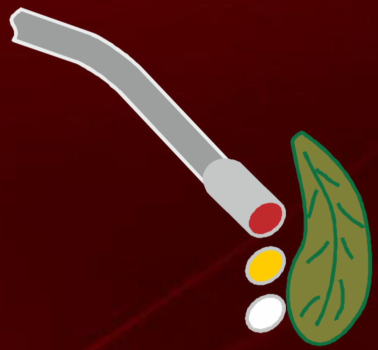
ΦPSII 0.478

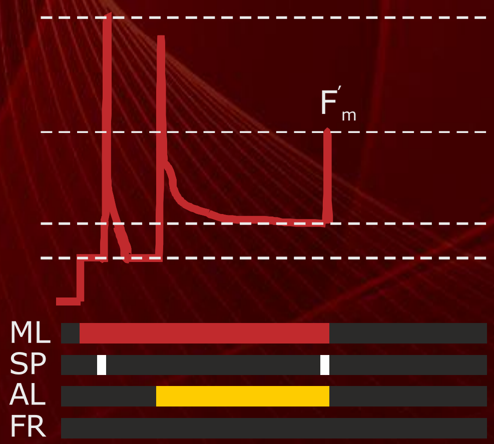
qP 0.628

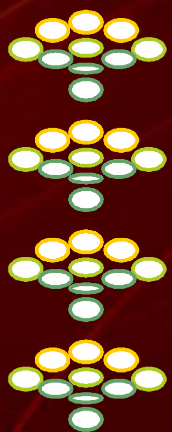
qN 0.442

Parameters of F_s and F_m' allow the calculation of the operational quantum yield efficiency of PSII (Φ_{PSII})





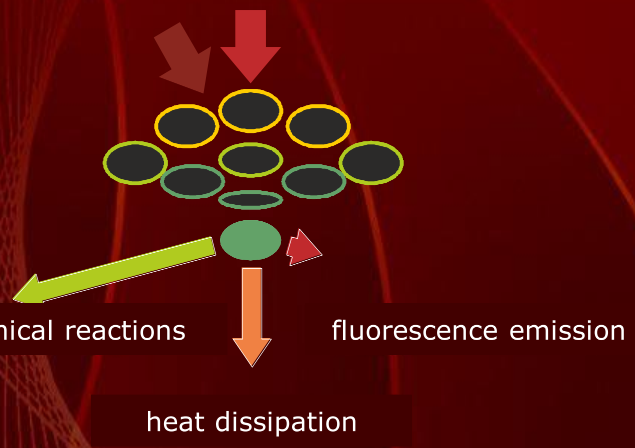


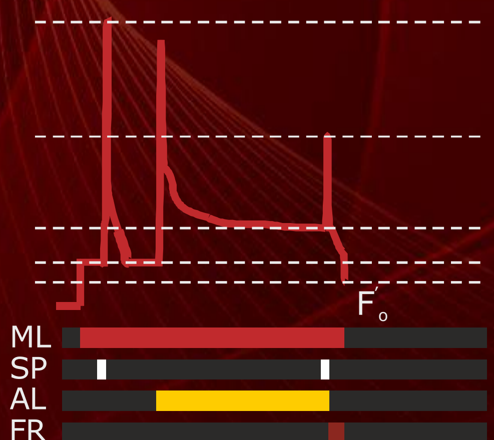


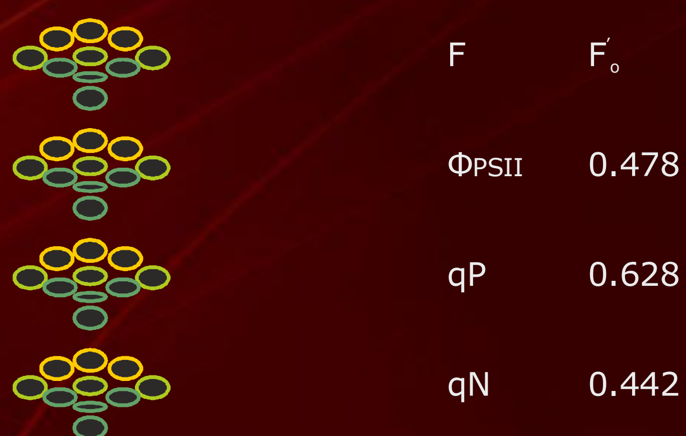
F	F'_m
ΦPSII	0.478
qP	0.628
qN	0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

Switching actinic light off and application of a far red illumination lowers fluorescence yield to a F_o' level which is the ‘in the light’ analogous of F_o but considerably lower.

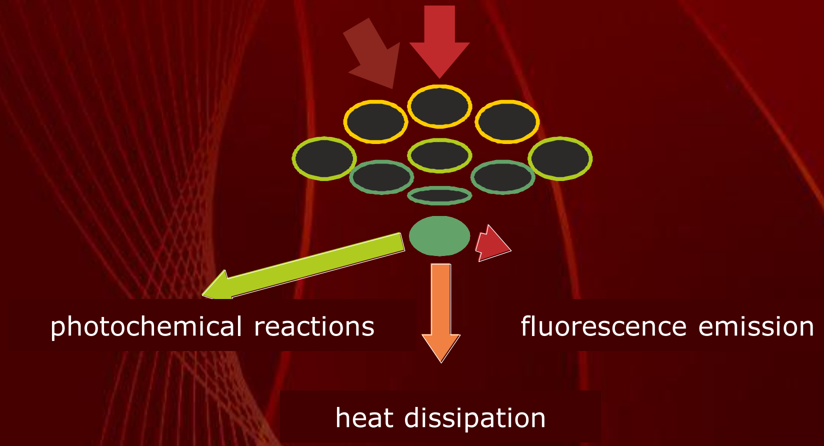


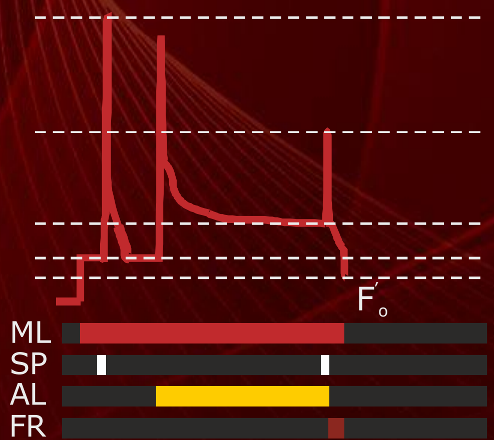


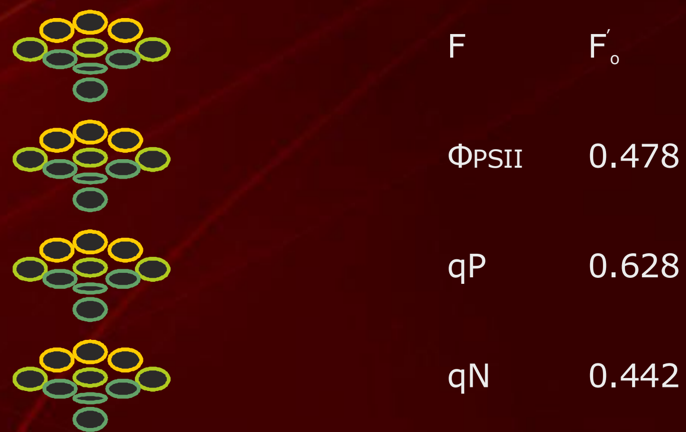


Analysis of Slow Kinetics Fluorescence Induction Curve

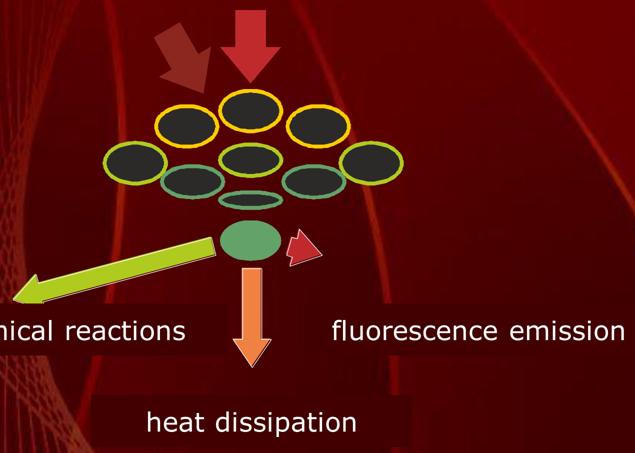
The decay of the F_o' levels is due to rearrangement of antenna components and engagement of heat-dissipation (non-photochemical quenching) mechanisms.

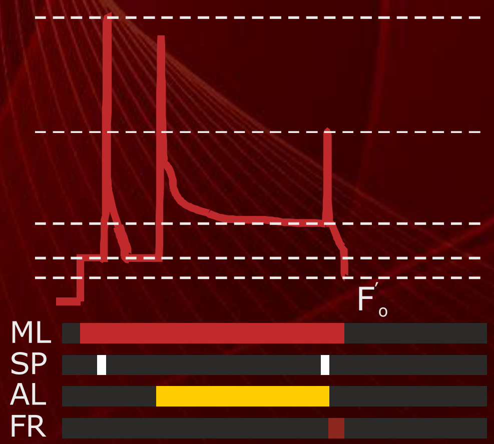






This is why we must use the F_o' level of fluorescence, rather than F_o, to calculate qP and qN quenching coefficients.

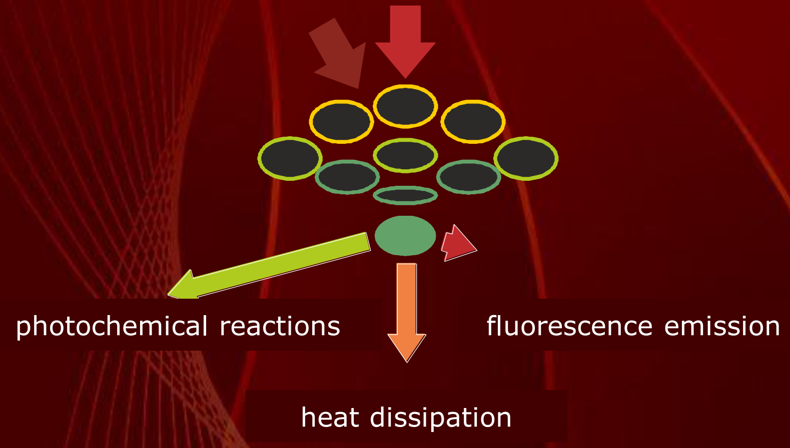


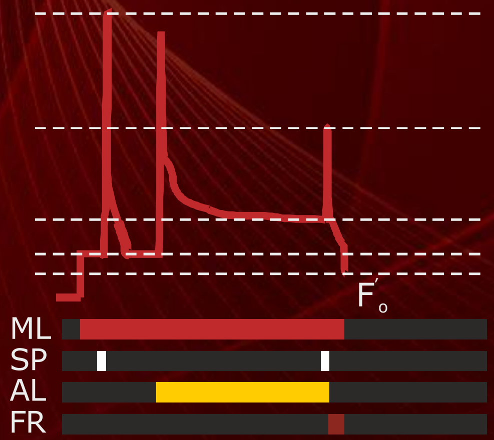


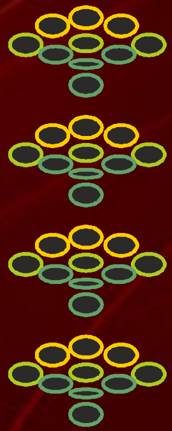
F	F_o'
Φ_{PSII}	0.478
qP	0.628
qN	0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

If direct measurement of F'_o is not possible we can use an approximation where $F_o' = F_o/[(F_v/F_m)+(F_o/F'_m)]$



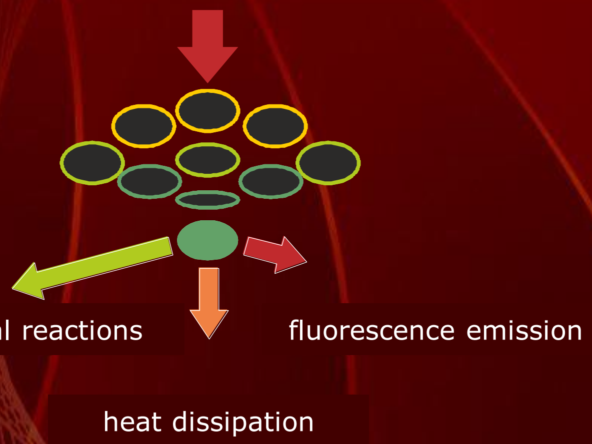


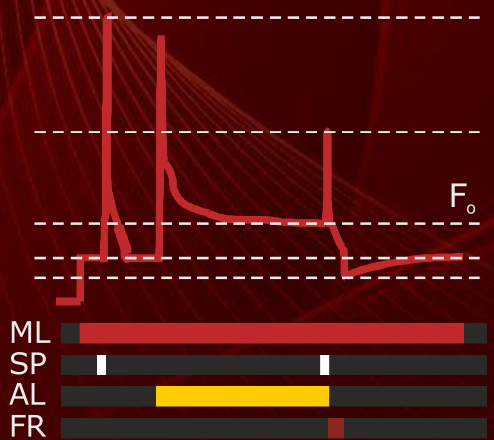


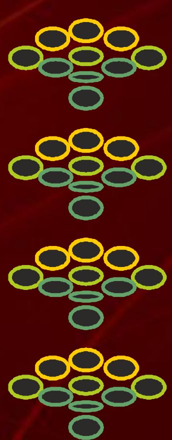
F	F'_o
ΦPSII	0.478
qP	>0.628
qN	<0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

In the dark, antenna components rearrangement and disengagement of non-photochemical processes completes, the basic fluorescence levels are restored back to F_o



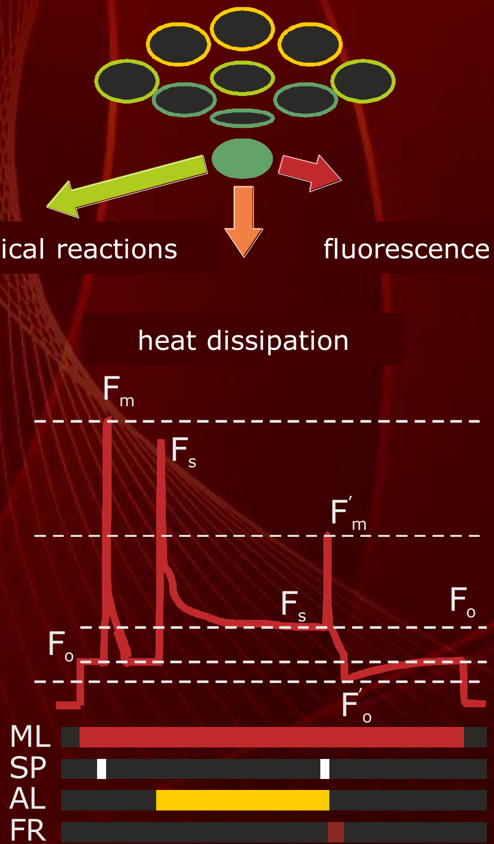


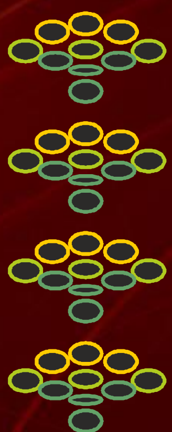


F	F_o
Φ_{PSII}	0.478
qP	>>0.628
qN	<<0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

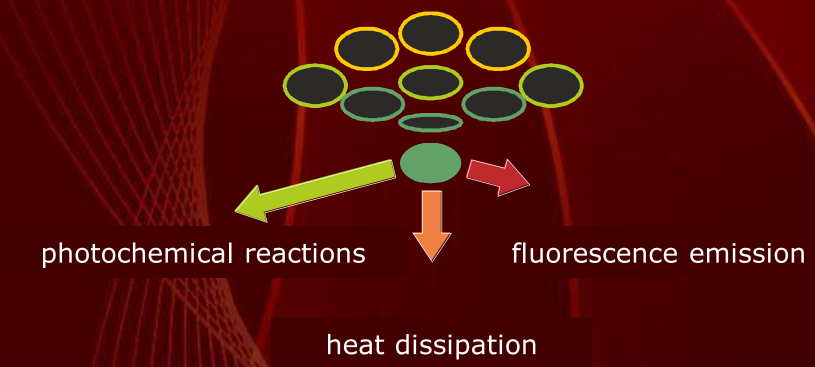
The qP and qN quenching coefficients allow a comprehensive examination of the photochemical processes

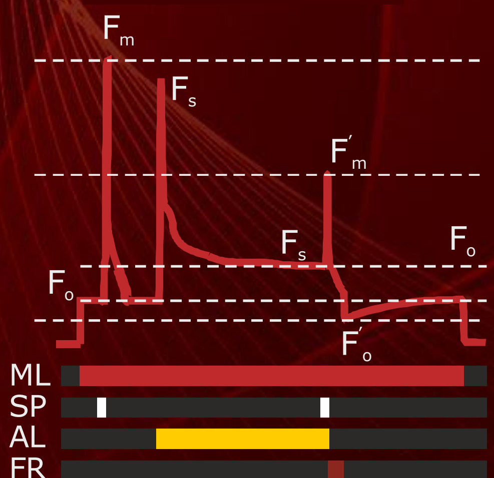


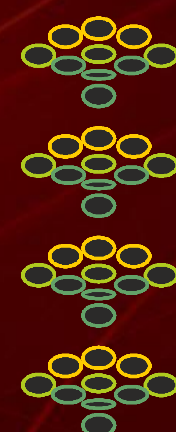


F	F_o
Φ_{PSII}	0.478
qP	>>0.628
qN	<<0.442

The qP parameter equals $(F'_m - F_t)/(F'_m - F'_o) = \Delta F / F'_v$



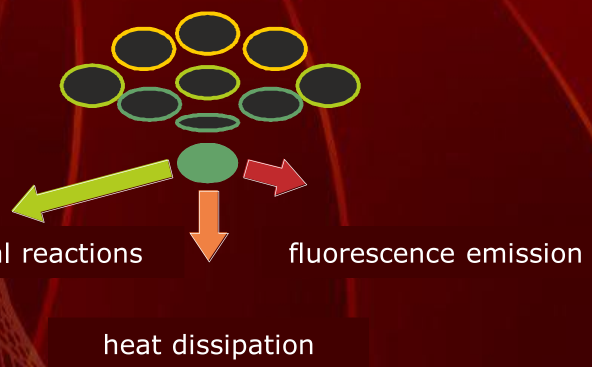


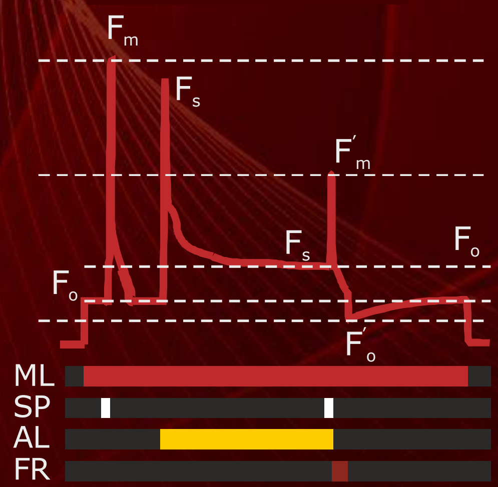


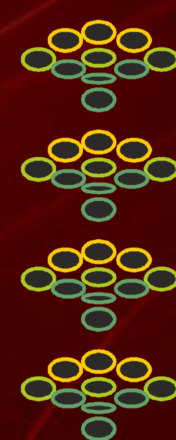
F	F_o
ΦPSII	0.478
qP	>>0.628
qN	<<0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

The qN parameter equals $(F_m - F'_m)/(F_m - F'_o)$. An almost equal expression is $1-(F'_m - F'_o)/(F_m - F_o) = 1-(F'_v/F_v)$

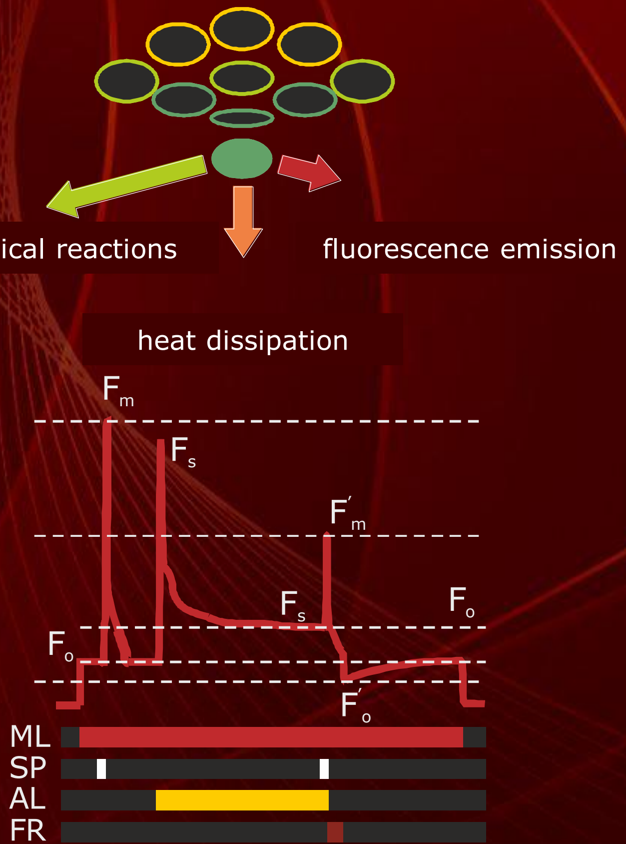


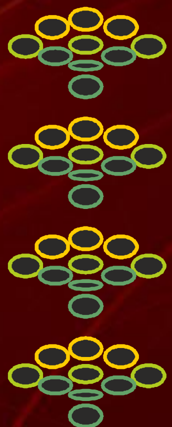




F	F_o
Φ_{PSII}	0.478
qP	>>0.628
qN	<<0.442

Another non-photochemical quenching parameter is the NPQ. This parameter is similar but not identical with the qN parameter but does not require knowledge of F'_o

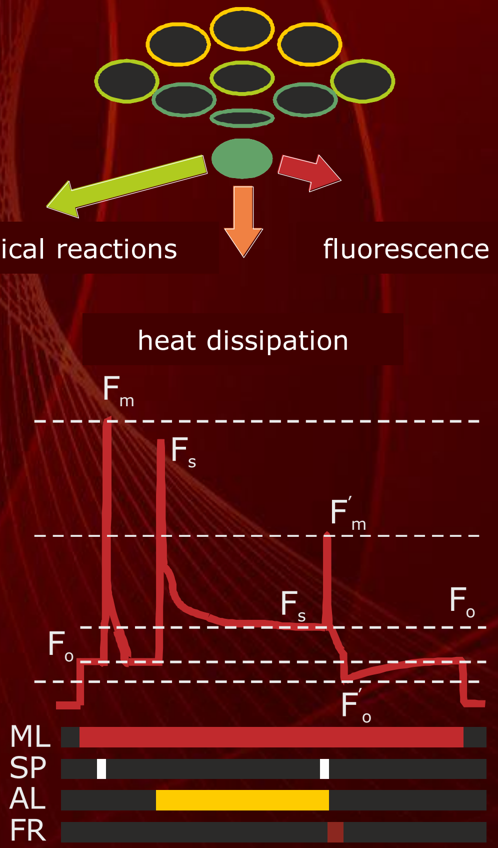


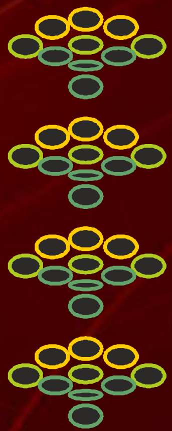


F	F_o
ΦPSII	0.478
qP	>>0.628
qN	<<0.442

Analysis of Slow Kinetics Fluorescence Induction Curve

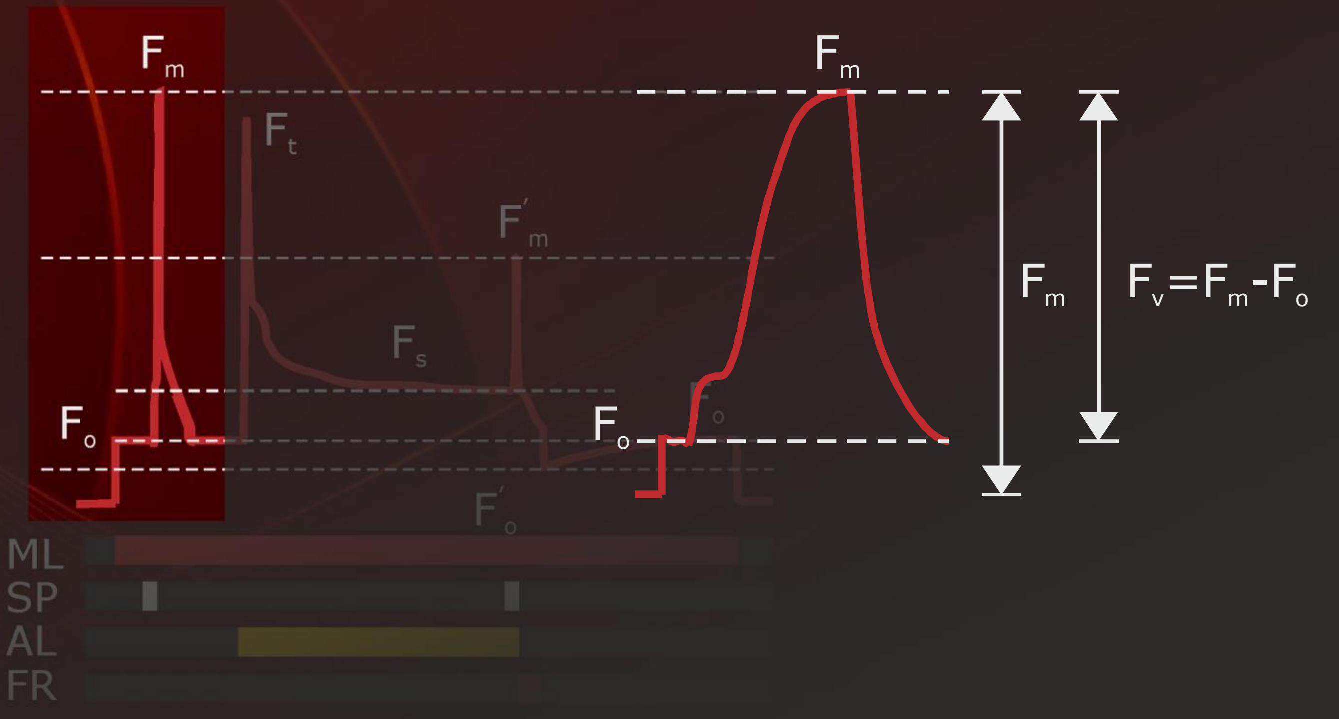
There is NPQ = $(F_m - F'_m)/F'_m$. However, this calculation of the NPQ parameter presupposes that any combination of determinations of fluorescence yields is characterized by similar fluorescence parameters 'in the dark'



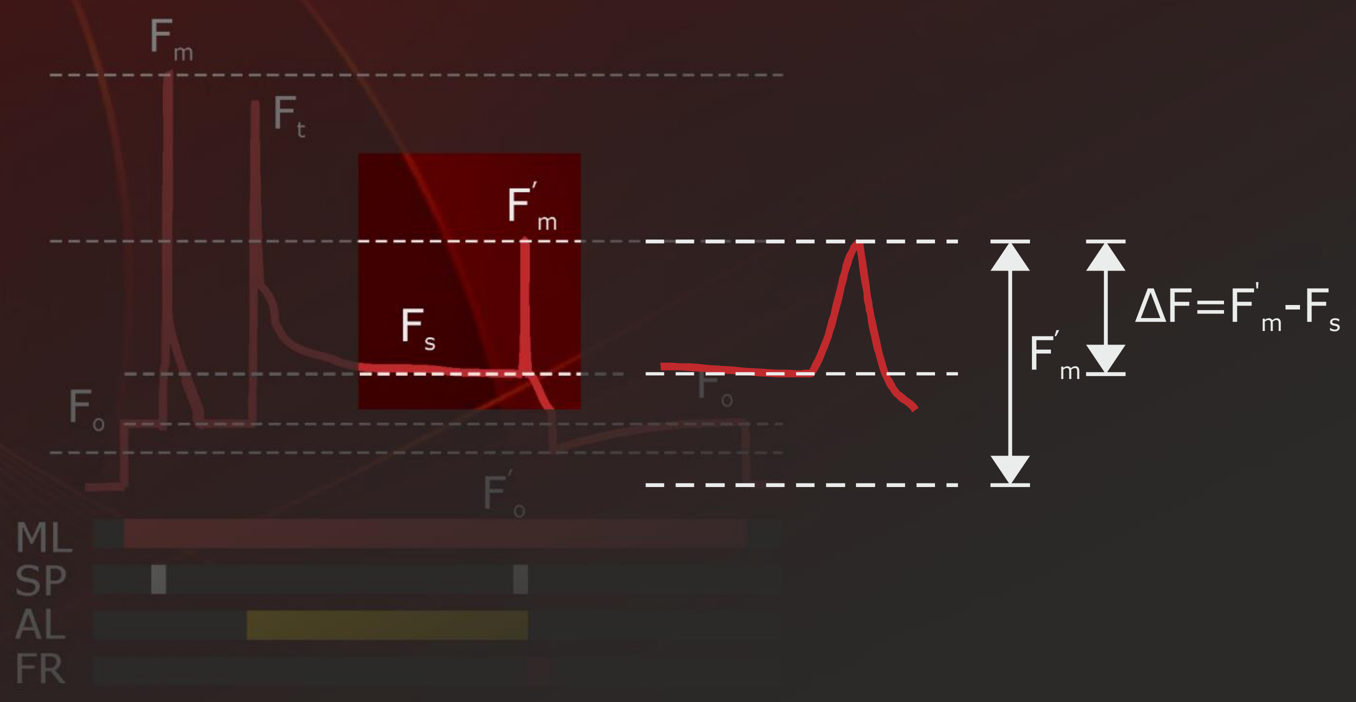


Analysis of Slow Kinetics Fluorescence Induction Curve

Maximum quantum yield of PS II photochemistry, $\Phi_{PSIIo}=F_v/F_m$, is a measure of the maximum percent of absorbed energy that can be used in photochemistry



Operational quantum efficiency of PS II photochemistry, $\Phi_{PSII}=\Delta F/F'_m$ is related to the photochemical work under a specific intensity of actinic light



Parameters of Slow Kinetics Fluorescence Induction Curve

F'_o, Basic fluorescence 'in the light'. Measures the operational efficiency of energy flow from antenna to the PSII reaction center.

F'_m, Maximum fluorescence 'in the light'. Is a measure of total energy flow through the PS II.

F'_v, Variable fluorescence 'in the light'. Is related to operational energy flow from PSII reaction center to the electron transport chain for a given light intensity.

$(\Delta F)/F'_m=\Phi_{PSII}$, PSII operational quantum efficiency. Measures the percent of energy that flows towards the electron transport chain to the total energy absorbed by PSII for a given light intensity.

Παράμετροι της καμπύλης βραδείας κινητικής φθορισμού

$qP=(F'_m-F_s)/(F'_m-F'_o)$, Photochemical quenching coefficient. Measures the percent of open PSII centers. The complementary measure, 1-qP, equals the percent of closed reaction centers and is also referred as the energy pressure or excitation pressure onto PS II.

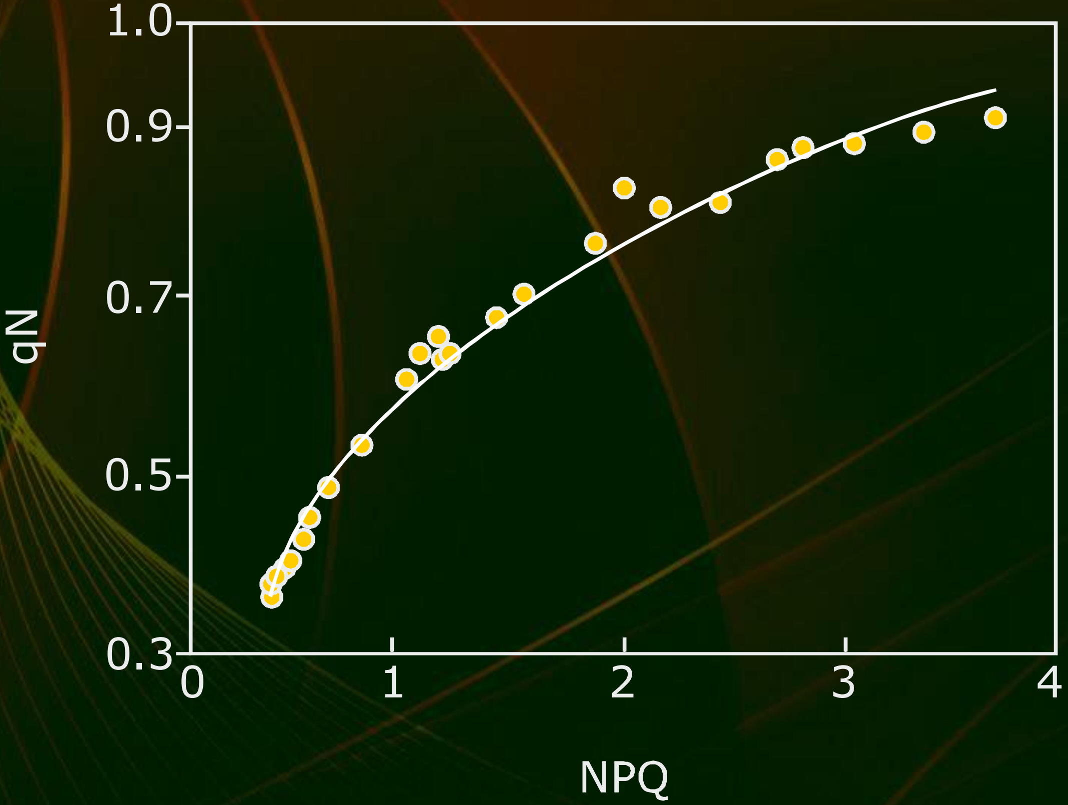
$qN=(F_m-F'_m)/(F_m-F'_o)$, Non-photochemical quenching coefficient. Measures the percent of PSII energy quenched by non-photochemical processes for a given light intensity.

$NPQ=(F_m-F'_m)/F'_m$, Non-photochemical quenching parameter (Stern-Volmer parameter). This is an alternative expression for measuring the percent of PSII energy quenched by non-photochemical processes for a given light intensity.

ETR=Φ_{PSII} × Q × f_1 × f_2: Apparent electron transport rate in the photochemical chain. Measures the rate of linear electron flow by using the quantum efficiency of PSII photochemistry and absorptance of incident radiation.

Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Είναι απολύτως ισοδύναμες οι δύο εκφράσεις της μη-φωτοχημικής απόσβεσης του φθορισμού (qN και NPQ);



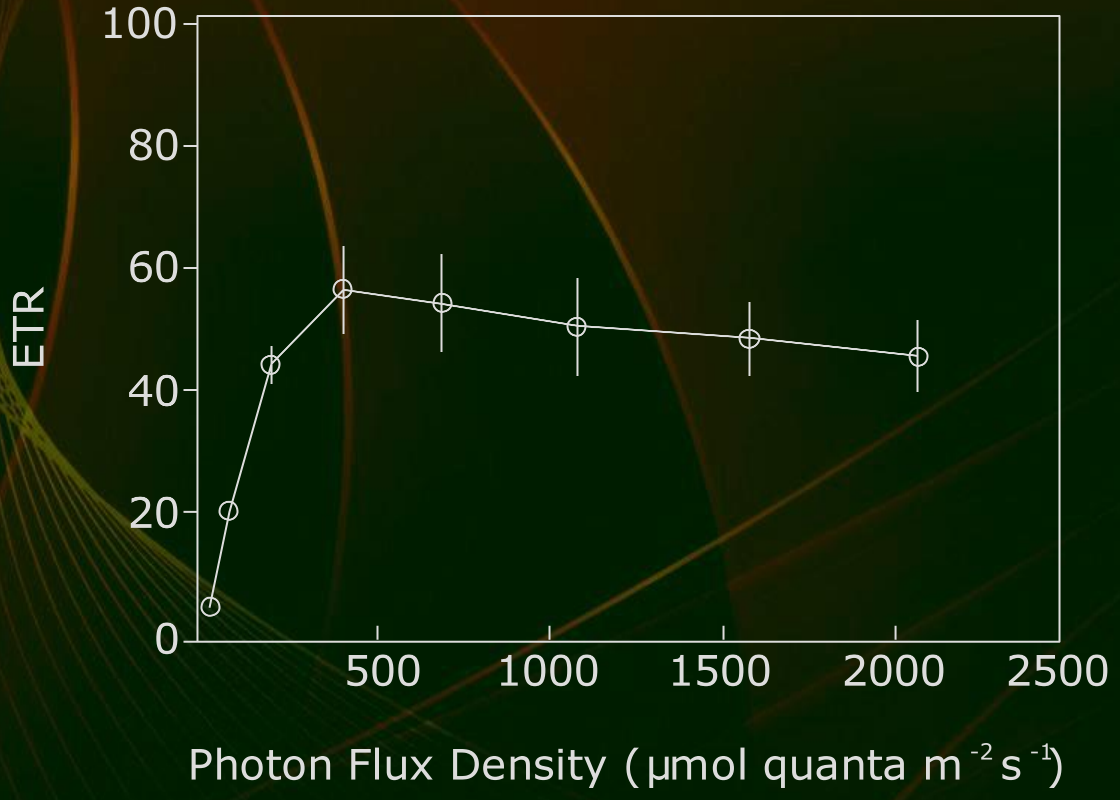
Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Είναι απολύτως ισοδύναμες οι δύο εκφράσεις της μη-φωτοχημικής απόσβεσης του φθορισμού (qN και NPQ);

Η σχέση μεταξύ qN και NPQ είναι θετική αλλά όχι γραμμική: οι δύο παράμετροι δεν είναι απόλυτα ισοδύναμες

Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Μπορούμε να χρησιμοποιούμε την παράμετρο ETR ως μέτρο της φωτοσυνθετικής ταχύτητας;



Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Μπορούμε να χρησιμοποιούμε την παράμετρο ETR ως μέτρο της φωτοσυνθετικής ταχύτητας;

Η παράμετρος ETR δίνει ένα μέτρο του ρυθμού επιτέλεσης των φωτεινών αντιδράσεων και υπό προϋποθέσεις σχετίζεται καλά με τη φωτοσυνθετική ταχύτητα

Υπολογίζεται ως $ETR = \Phi_{PSII} \times Q \times f_1 \times f_2$ όπου:

Q: ένταση προσπίπτουσας ακτινοβολίας (PAR)

f_1: συντελεστής κατανομής φωτονιακής ενέργειας στο PS II (τυπικά ίσος με 0,5)

f_2: συντελεστής απορροφητικότητας φύλλου (τυπικά χρησιμοποιείται η τιμή 0,84)

Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Μπορούμε να χρησιμοποιούμε την παράμετρο ETR ως μέτρο της φωτοσυνθετικής ταχύτητας;

Προϋποθέσεις για την χρήση του ETR:

1) Ο συντελεστής f_2 είναι γνωστός ή υπολογίζεται μέσω σφαίρας ολοκλήρωσης
2) Ο συντελεστής f_2 είναι παρόμοιος μεταξύ δειγμάτων τα οποία συγκρίνονται ως προς το ETR

Προϋποθέσεις για την χρήση του ETR ως μέτρο της φωτοσυνθετικής ταχύτητας:

3) Η συμβολή των εναλλακτικών οδών κατανάλωσης της ενέργειας των φωτεινών αντιδράσεων είναι σταθερή
   - Ως εναλλακτικές οδοί ορίζονται η φωτοαναπνοή και η αντίδραση Mehler
   - Υπό συνθήκες καταπόνησης και ιδιαίτερα σε υψηλές εντάσεις ακτινοβολίας η συμβολή των παραπάνω οδών απόσβεσης της ενέργειας μεγαλώνει

Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

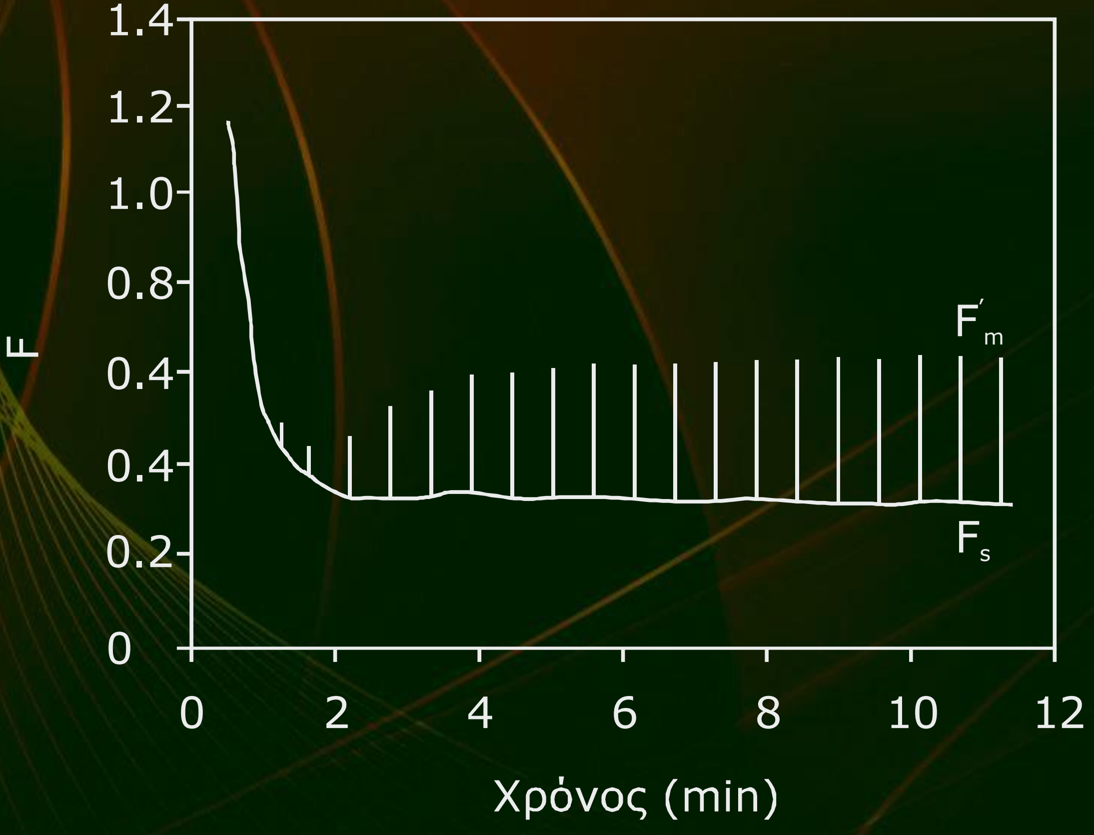
Πως γνωρίζουμε ότι ένα δείγμα έχει φθάσει σε σταθερή κατάσταση για δεδομένη ένταση προσπίπτουσας ακτινοβολίας ώστε να προβούμε σε μέτρηση της φωτοχημικής ικανότητας;

Η απόκτηση της σταθερής κατάστασης σχετίζεται με την ολοκλήρωση της ‘επαγωγής της φωτοσύνθεσης’ η οποία με τη σειρά της περιλαμβάνει σταθεροποίηση των φωτεινών αντιδράσεων, δραστηριοποίηση του κύκλου Calvin-Benson και της στοματικής αγωγιμότητας

Πληροφορίες για τον χρόνο απόκτησης της σταθερής κατάστασης προσφέρει η λεγόμενη **καμπύλη επαγωγής**

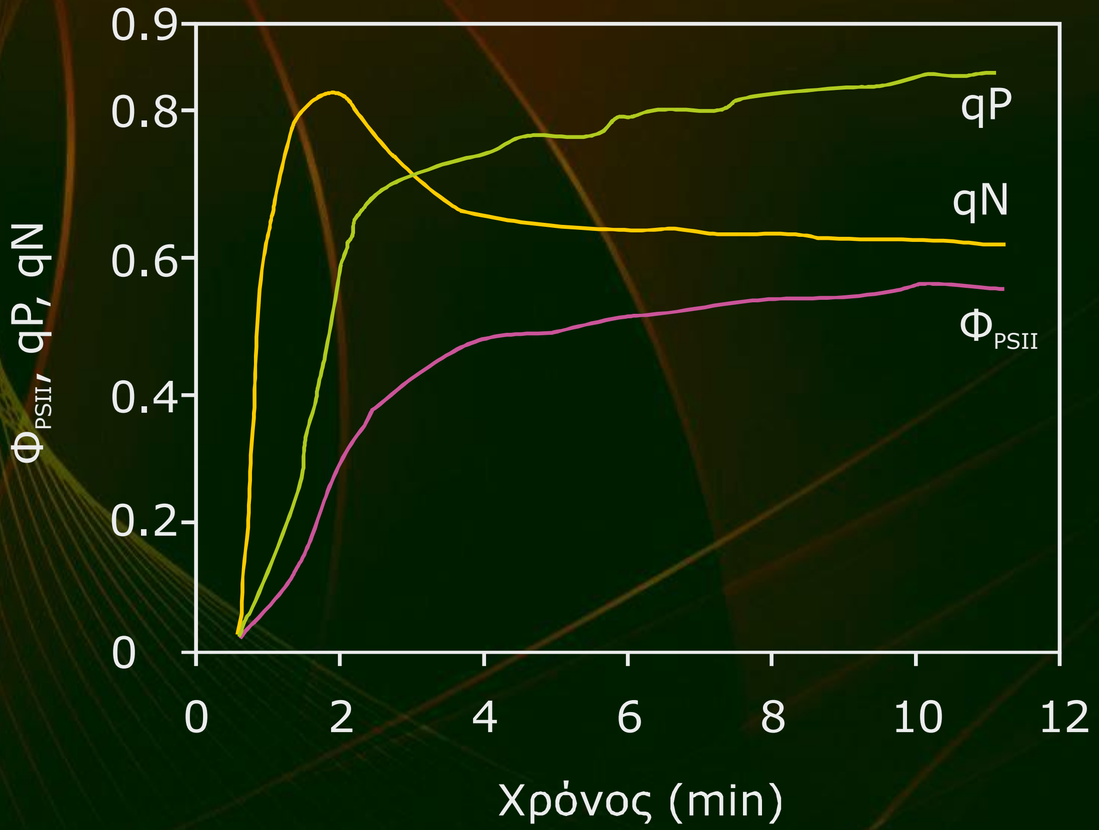
Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Πως γνωρίζουμε ότι ένα δείγμα έχει φθάσει σε σταθερή κατάσταση για δεδομένη ένταση προσπίπτουσας ακτινοβολίας ώστε να προβούμε σε μέτρηση της φωτοχημικής ικανότητας;



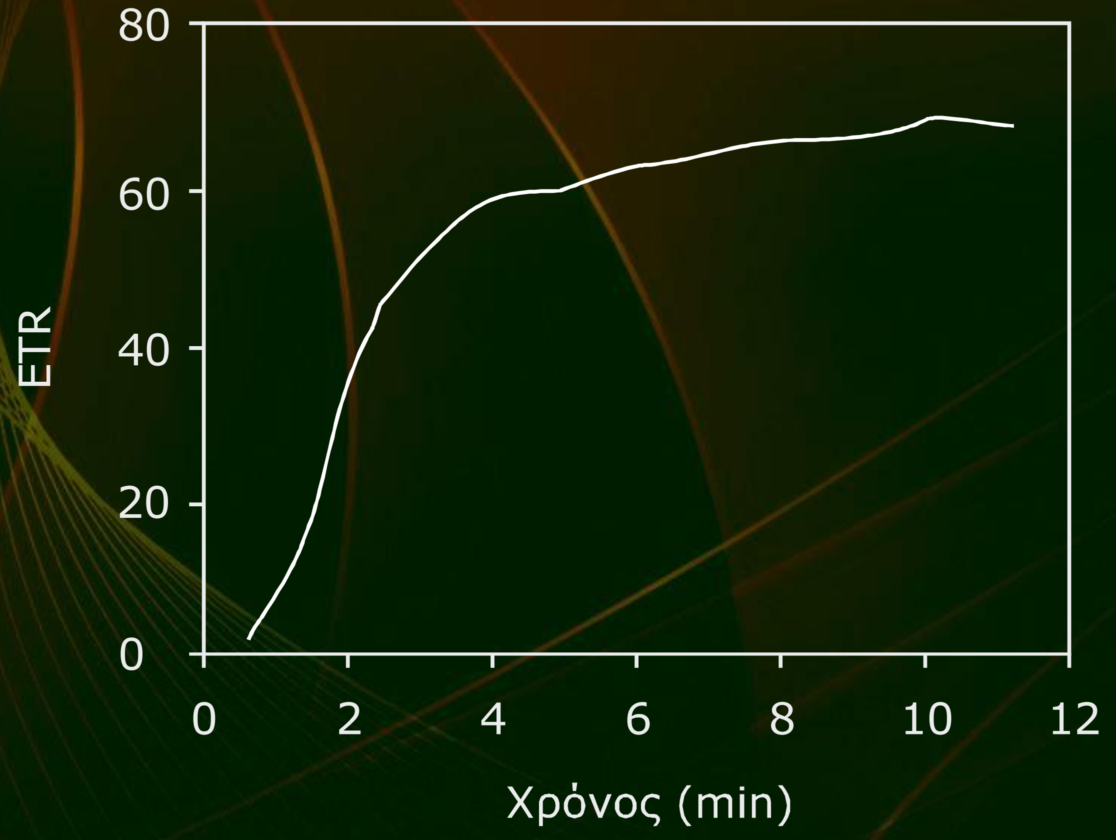
Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Πως γνωρίζουμε ότι ένα δείγμα έχει φθάσει σε σταθερή κατάσταση για δεδομένη ένταση προσπίπτουσας ακτινοβολίας ώστε να προβούμε σε μέτρηση της φωτοχημικής ικανότητας;



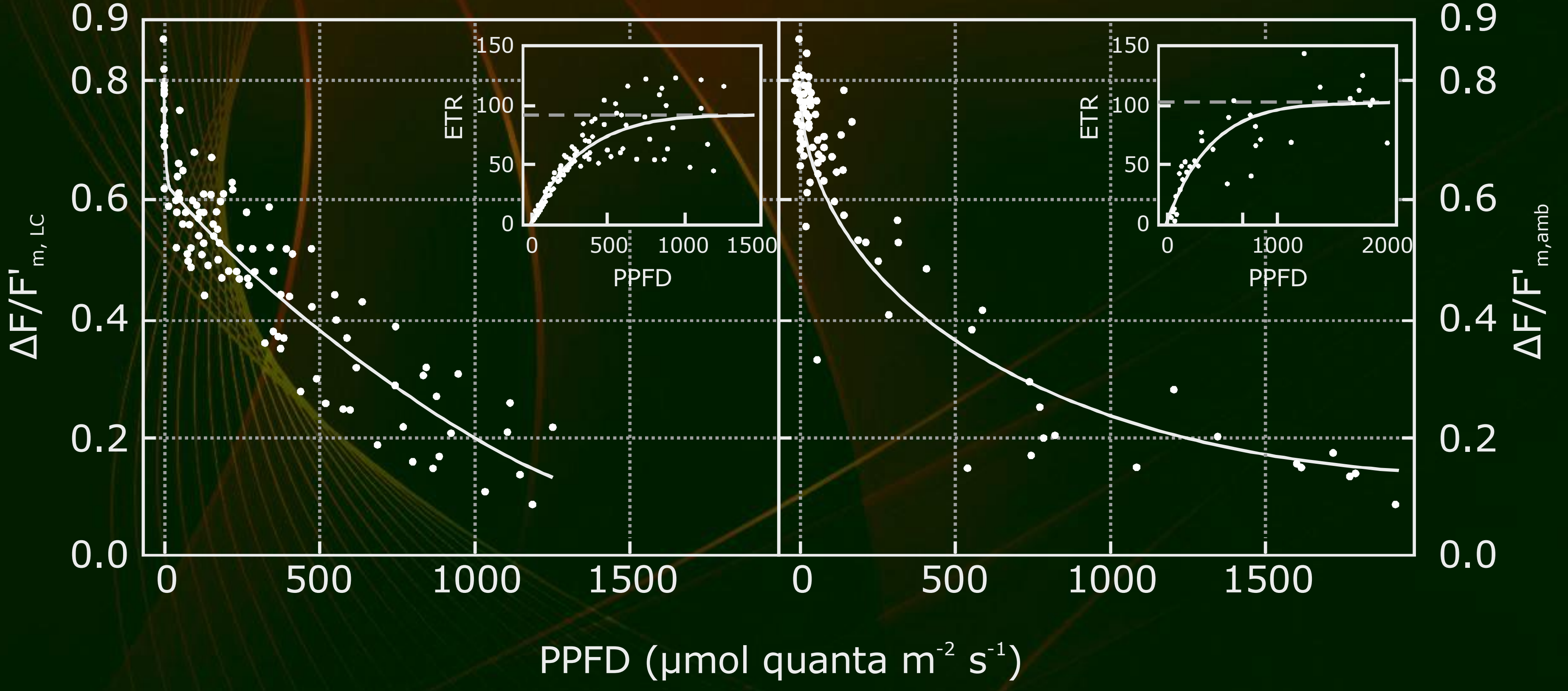
Πρακτικά ζητήματα σχετικά με την καμπύλη ταχείας κινητικής

Πως γνωρίζουμε ότι ένα δείγμα έχει φθάσει σε σταθερή κατάσταση για δεδομένη ένταση προσπίπτουσας ακτινοβολίας ώστε να προβούμε σε μέτρηση της φωτοχημικής ικανότητας;

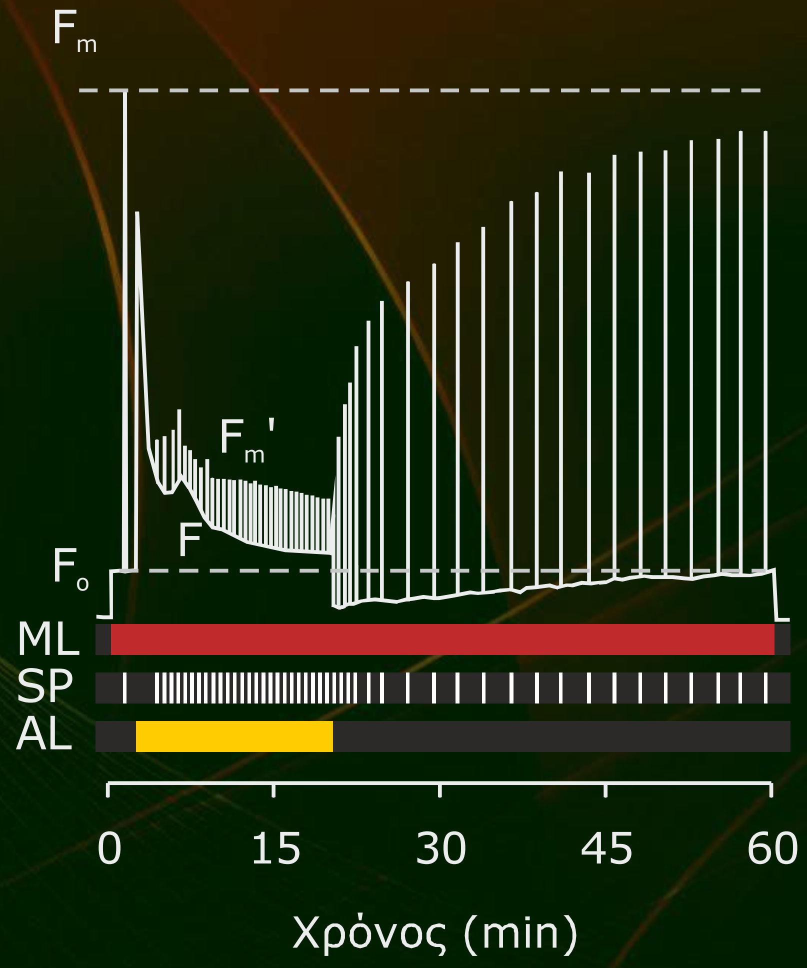


Τι είναι οι καμπύλες φωτός και ποιες πληροφορίες μας παρέχουν; Πως καταγράφεται μια καμπύλη φωτός;

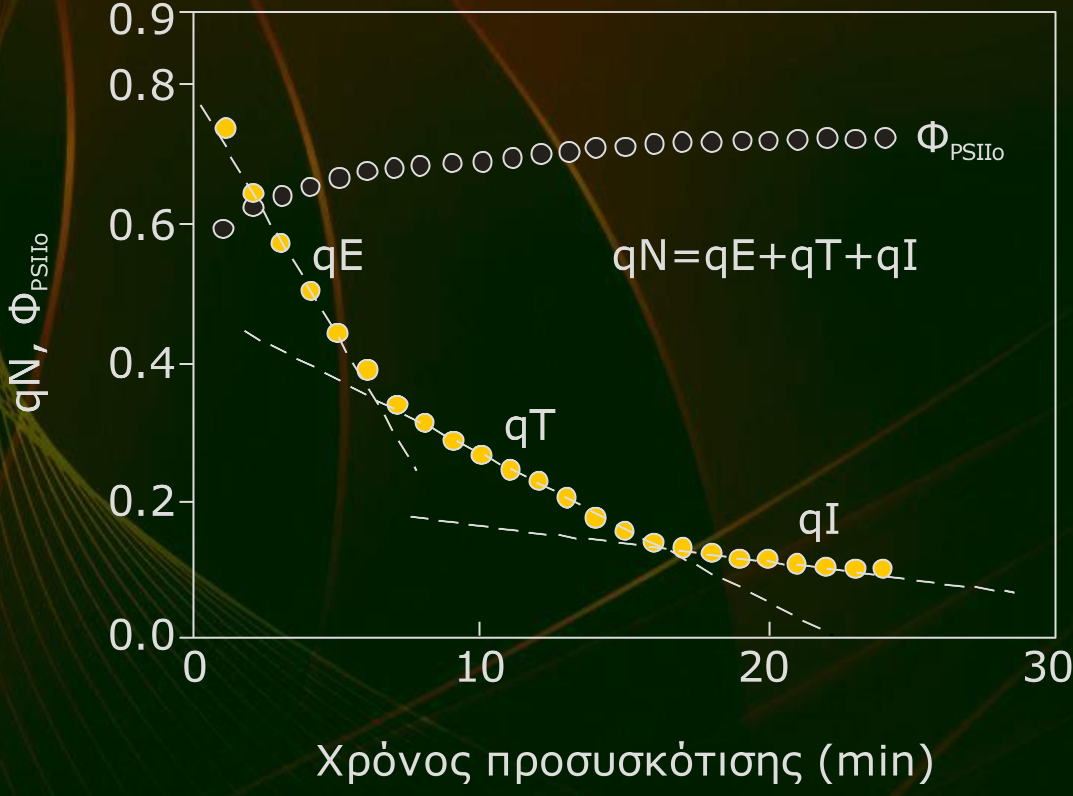
Ποιες είναι οι διαφορές ανάμεσα σε ταχείες καμπύλες φωτός (rapid light curves) και καμπύλες φωτός περιβάλλοντος (ambient light curves);



Τι είναι η κινητική φθορισμού στο σκοτάδι (dark relaxation kinetics);



Τι πληροφορίες μας δίνει και πως ερμηνεύεται η μορφή της κινητικής φθορισμού στο σκοτάδι;

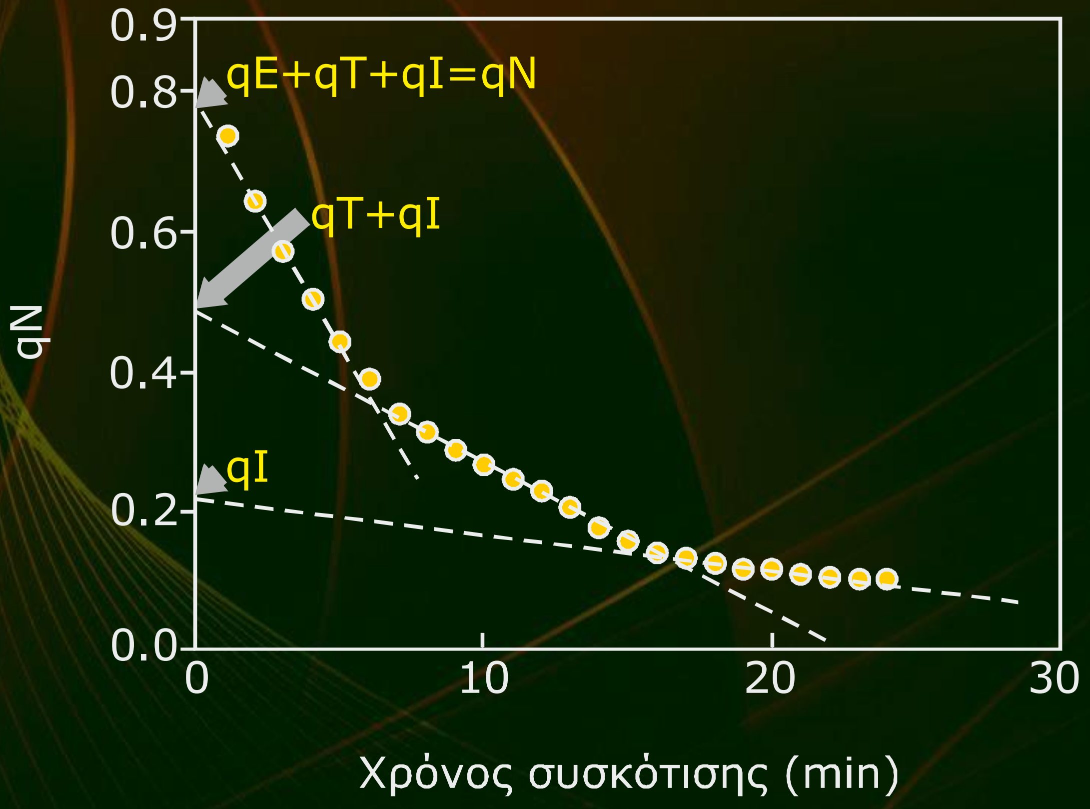


Πρακτικά ζητήματα: κινητική στο σκοτάδι

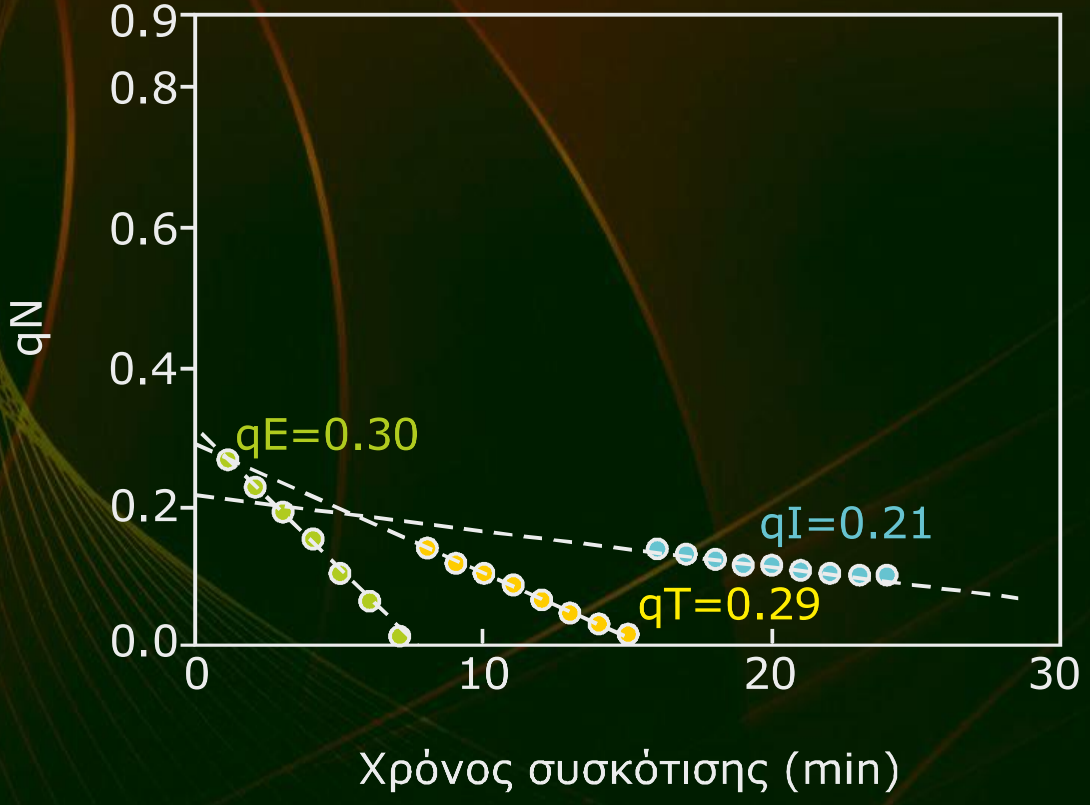
Τι πληροφορίες μας δίνει και πως ερμηνεύεται η μορφή της κινητικής φθορισμού στο σκοτάδι;

Η μελέτη της κινητικής στο σκοτάδι (dark relaxation kinetics) προσφέρει στοιχεία για την σχετική συνεισφορά των βασικών μη φωτοχημικών-φωτοπροστατευτικών μηχανισμών (μηχανισμών απόσβεσης της πλεονάζουσας ενέργειας διέγερσης μέσω μη ακτινοβολούσας αποδιέγερσης, qN) δηλ. του **κύκλου των ξανθοφυλλών** (**qE**) και της **κατάστασης μετάπτωσης** (**qT**). Επίσης δίνει μια εκτίμηση της έκτασης της ελεγχόμενης και μη ελεγχόμενης (λόγω βλαβών των φωτοσυστημάτων) **φωτοαναστολής της φωτοσύνθεσης** (**qI**)

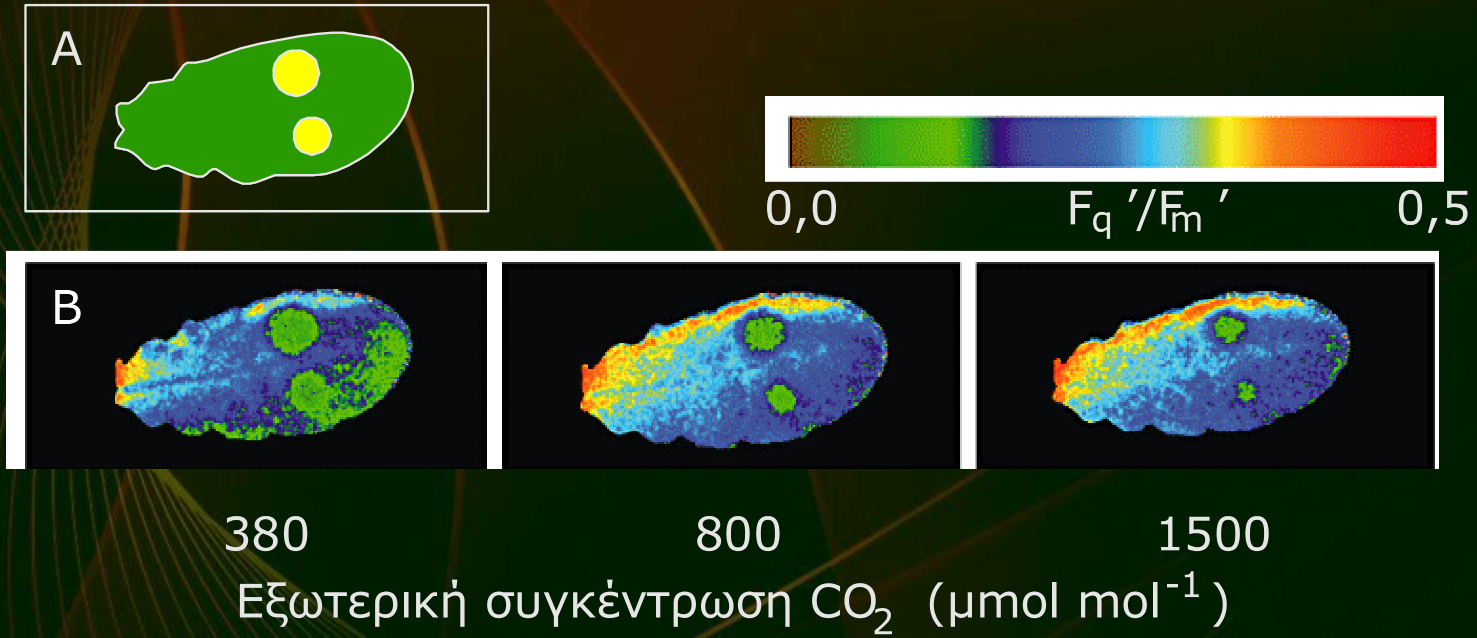
Σε ποιες συνιστώσες αναλύεται η μη-φωτοχημική απόσβεση και πως οι συνιστώσες αυτές ηρεμούν στο σκοτάδι;



Σε ποιες συνιστώσες αναλύεται η μη-φωτοχημική απόσβεση και πως οι συνιστώσες αυτές ηρεμούν στο σκοτάδι;

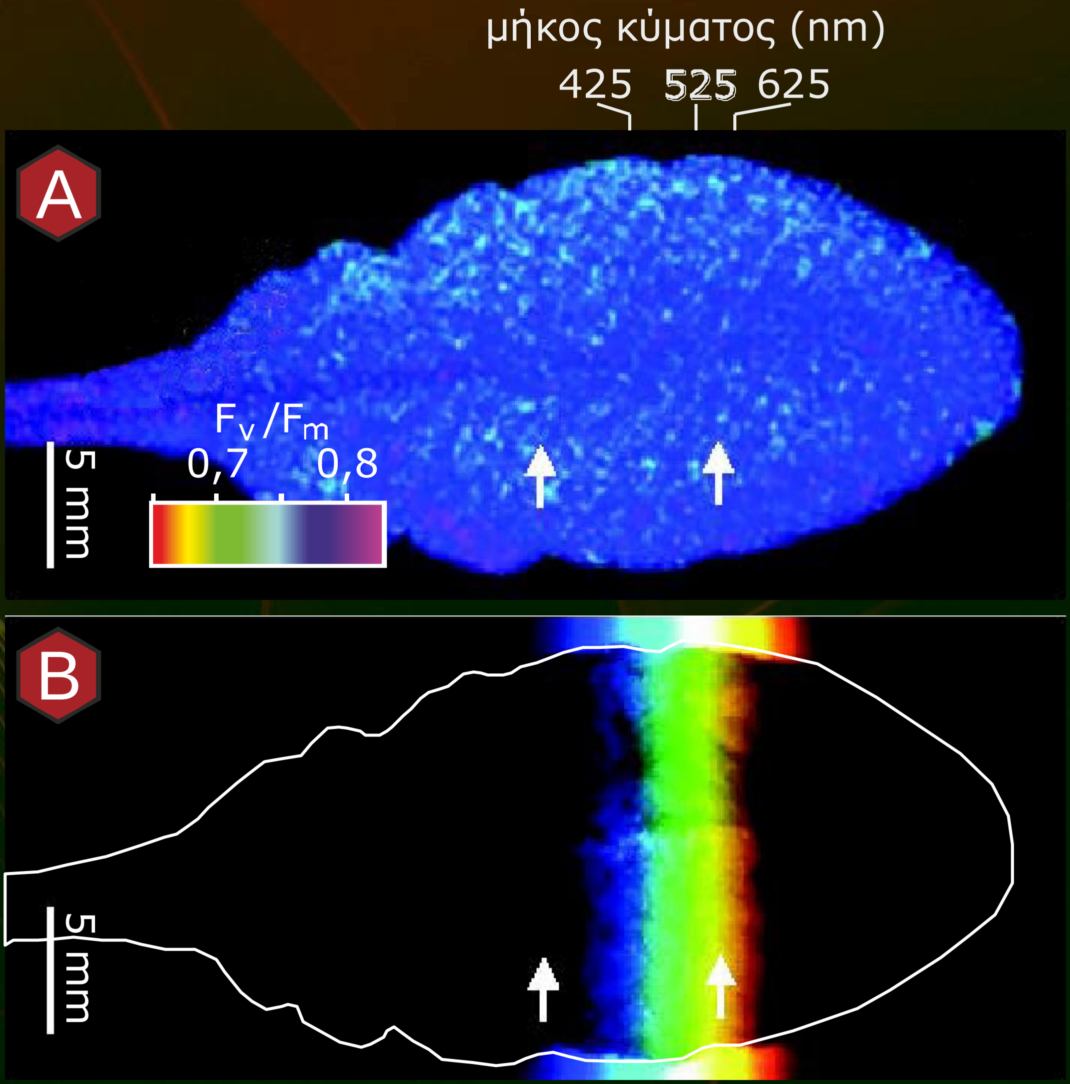


Απεικονιστική φθορισμομετρία



Παραλλαγές της τεχνικής της φθορισμομετρίας χλωροφύλλης

Απεικονιστική φθορισμομετρία



Παραλλ… χλωροφύλλης

Απεικονιστικ…