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作物生理生态研讨会暨光合作用研究技术培训班

WALZ

调制叶绿素荧光仪(PAM)实验技术与应用

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中国区技术服务中心

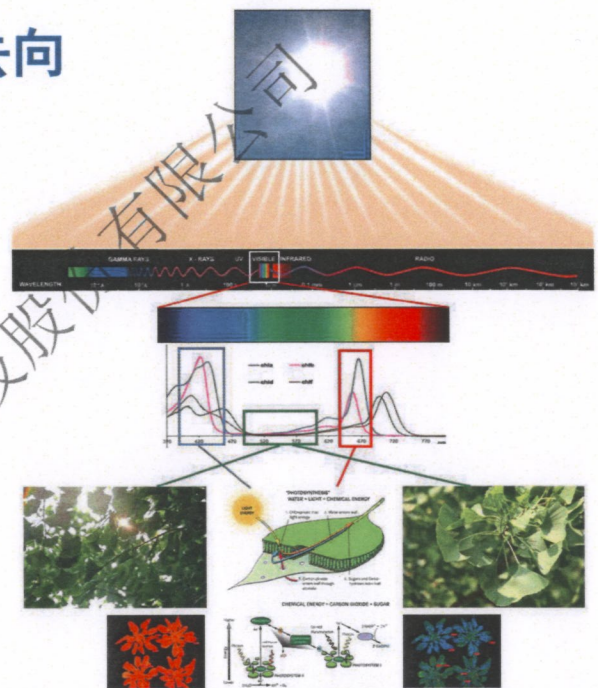
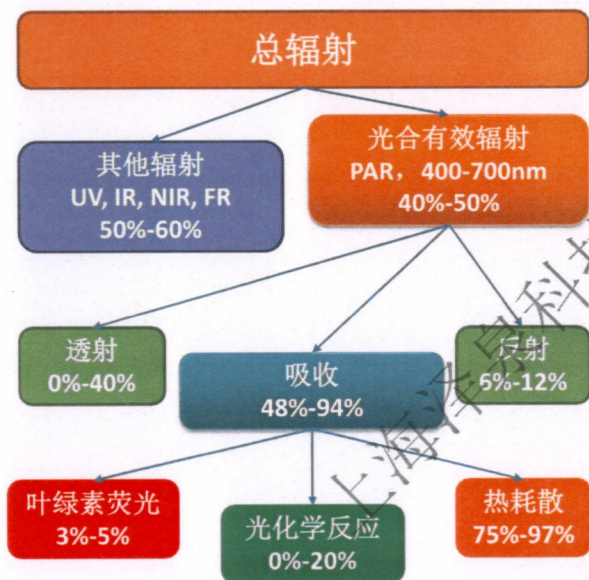


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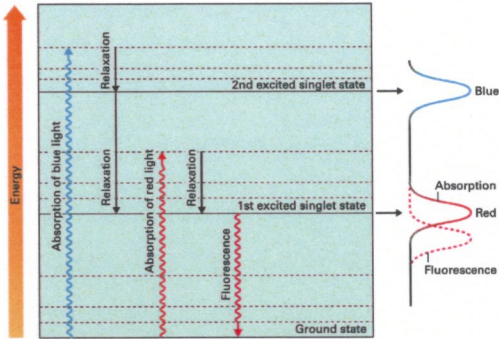
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光合作用能量转换与叶绿素荧光

光合作用的能量来源与去向



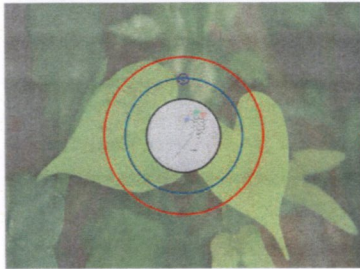
光能捕获与叶绿素荧光



当叶绿素a分子吸收光时，它会从其基态激发到单线激发态 (Chl*)

激发态叶绿素分子可以通过以下三种途径返回基态：

1. 激发能可以转移到反应中心以驱动光合作用 (光化学, P)
2. 可以作为热量释放 (热耗散, H)
3. 能量可以重新发射为波长更长的光 (叶绿素荧光, F)



光化学(P)
叶绿素荧光(F)
(波长变长)
热耗散(H)

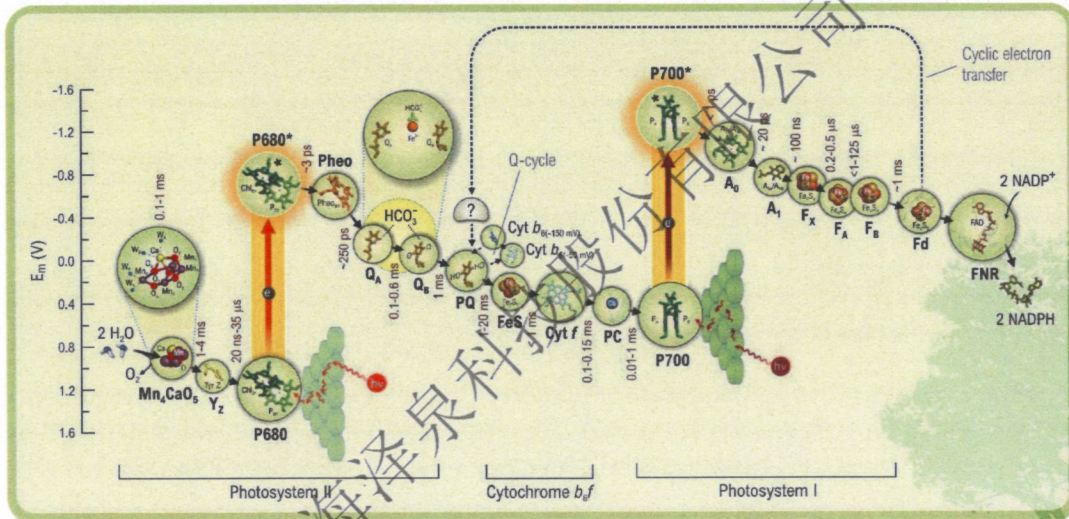
激发能中的一小部分重新以光的形式发射

叶绿素荧光



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叶绿素荧光主要指的是PSII的Chla荧光



PS II是一个相对缓慢的反应中心(电荷分离的可逆性)，而PS I是一个较快的反应中心(电荷分离的不可逆性)。相对缓慢的反应中心会使激发能寿命的延长，累积的激发能以荧光释放的比例更大。



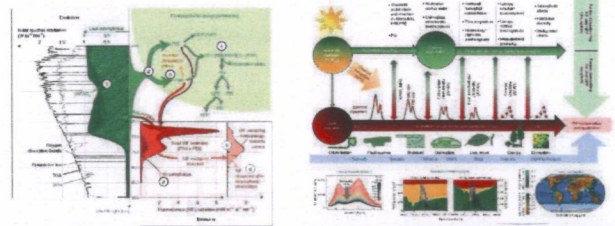
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光能传递与叶绿素荧光产生的相关时域

- 飞秒(fs)时域可以测量chl_b和chl_a之间的能量传递
- 皮秒(ps)-纳秒(ns)时域可以获得关于PSII天线内的能量平衡的信息
- **亚微秒(Sub-μs)时域主要探测与PSII天线相关的过程**
- 微秒(μs)时域开始反映光合作用电子传递链中的电子传递反应
- 毫秒(ms)时域可以探测整个电子传递链(长达200-1000ms)
- 分钟(min)级时域则可以反应电子传递链和卡尔文循环活性之间的平衡(稳态)
- 日-月-年级时域可以反映生态系统, 地球环境科学的初级生产力等 (GPP/NPP)



Chlorophyll a fluorescence illuminates a path connecting plant molecular biology to Earth-system science

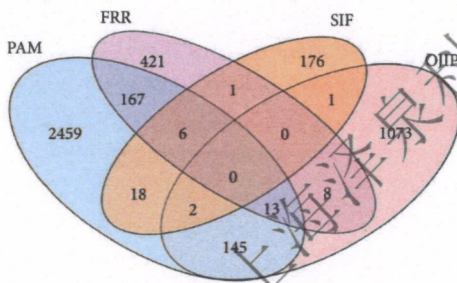


Simple Experiments with the JUNIOR-PAM Chlorophyll Fluorometer

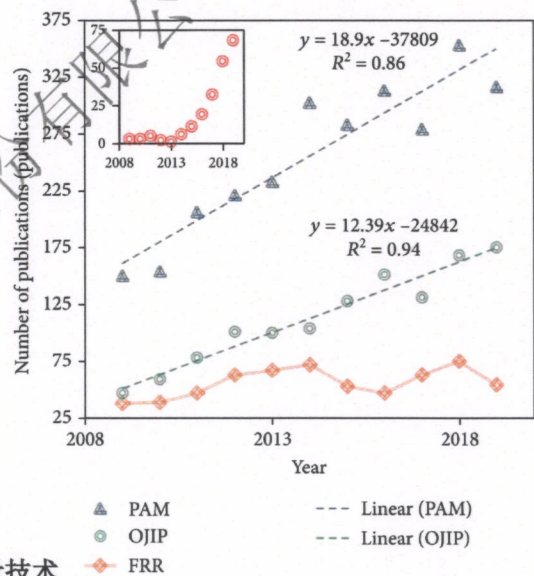
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常用的叶绿素荧光的测量方法与工具

- PAM:** 脉冲幅度调制荧光
- OJIP:** 快速荧光动力学
- FRR:** 快速重复率荧光
- SIF:** 日光诱导荧光



PAM仍是目前最受欢迎和最具影响力的叶绿素荧光测量技术



<https://doi.org/10.34133/2020/6293145>

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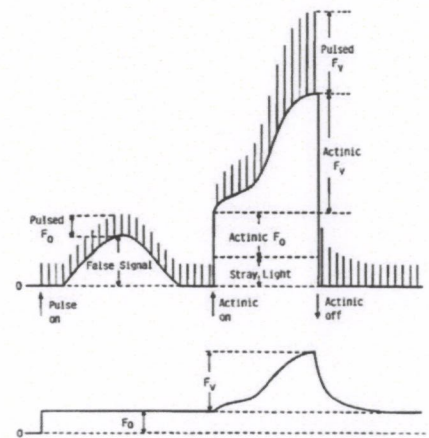
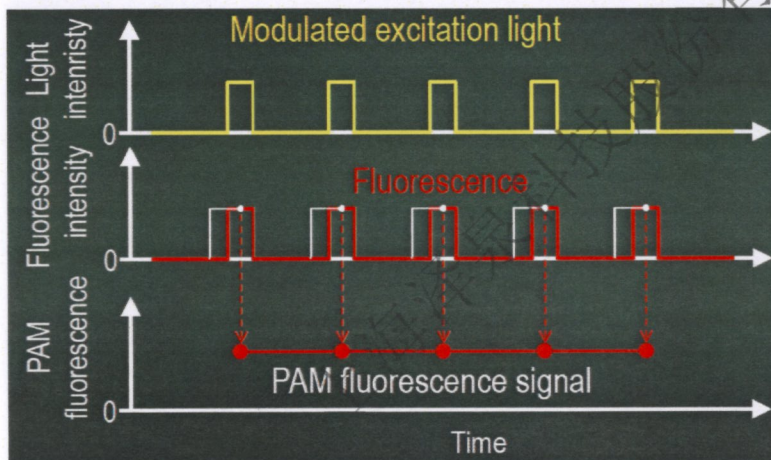
脉冲振幅调制(PAM)叶绿素荧光

脉冲振幅调制(PAM)叶绿素荧光

PAM测量光 (Measuring Light, ML)：用于叶绿素荧光信号的激发和检测。

PAM荧光是搭载了PAM (Pulse Amplitude Modulation)技术的叶绿素荧光仪。

PAM测量叶绿素荧光时只检测由恒定高度的 μs 级测量光脉冲引起的荧光幅度。



PAM测量光(ML)的功能和属性

PAM测量光 (Measuring Light, ML)：用于叶绿素荧光信号的激发和检测。

强度Intensity, Int.

单次测量光脉冲的光量子密度，测量光强度的增加可以显著增加荧光信号的水平

频率Frequency, Freq.

单位时间测量光脉冲闪烁的次数，测量光频率的增加可以显著提升荧光信号的信噪比

只有测量光时，测量脉冲的积分光强非常低，仅具有非常微弱的稳定光化效应。

激发速率始终保持在 Q_A 向 Q_B 转移电子的速率以下， Q_A 全部处于氧化态，测到一个荧光强度 F_0

F_0 是PSII仍处于还原状态(PSII反应中心仍处于开放状态)时的荧光发射量。

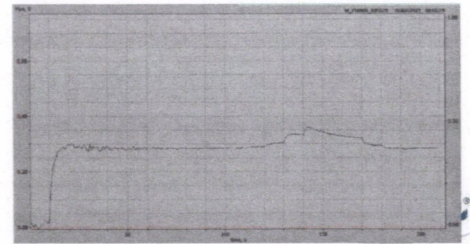
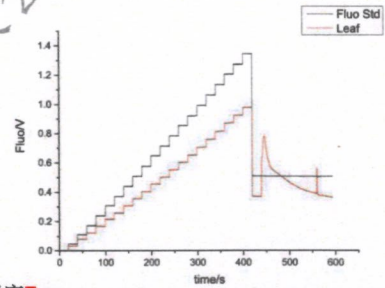
一个好的测量光强度的关键标准是诱导出稳定的荧光强度。

测量光的积分强度由测量光强度(Int.)和频率(Freq.)决定

只打开测量光的情况下，测量光频率可以很低

打开光化光或执行饱和脉冲时，测量光频率要高 (Auto MF-High)

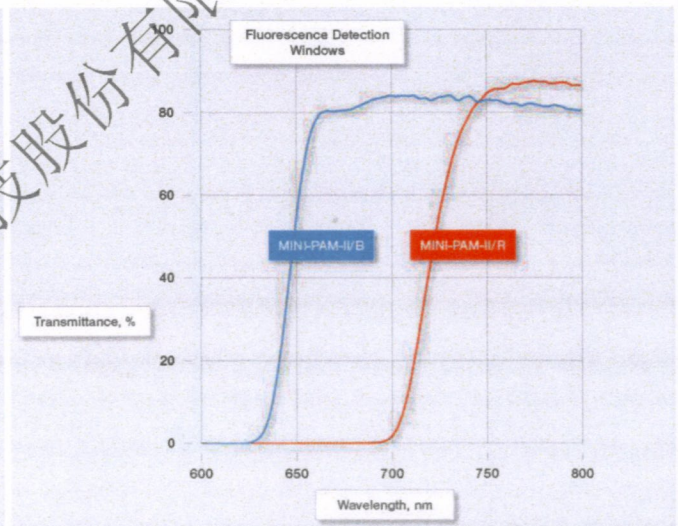
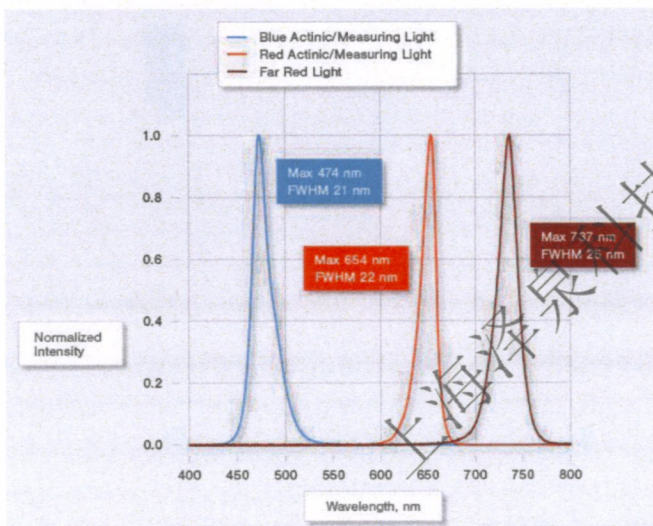
高频测量光可以显著提升信噪比！高频测量光具有更强的光化学效应！



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PAM检测器的功能和属性

PAM测量光(Measuring Light, ML)的波长与信号检测

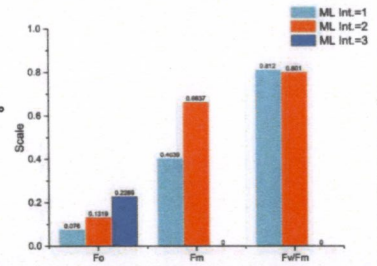


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PAM测量光强度-信号标度-检测器的关系

非常重要，因为检测器有上限，PAM测量通常要求测量前将Ft调至一定范围

测量光强度低，荧光寿命短，信号低，可变荧光/本底荧光 $>5-6$ ，PSII的量子效率被高估。
测量光强度高，荧光寿命长，信号高，最大荧光超过检测上限，PSII的量子效率被低估。
测量光强度过高就会有明显的光化学效应。



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PAM光化光(AL)和饱和脉冲(SP)的功能和属性

PAM光化光 (Actinic Light, AL)：用于诱导植物发生光合作用

PAM荧光仪内置的稳定光源，具有强度可调节的属性，每个强度档位对应相对固定的PAR。

使用PAM内置光源作为光化光诱导光合作用时，必须对光化光的强度非常明确！

光强校准：仪器或软件——样品水平实际光强——输出数据——样品真实生理反应，明确统一。

PAM饱和脉冲 (Saturation Pulse, SP)：用于关闭所有反应中心，计算数据

饱和脉冲是叶绿素荧光分析方法的关键要素，其定义决定了它具有光照强度和长度双重属性。

饱和脉冲的理想光照强度应该足够强，能使光合作用饱和，也就是高于光饱和点以上甚至更高。

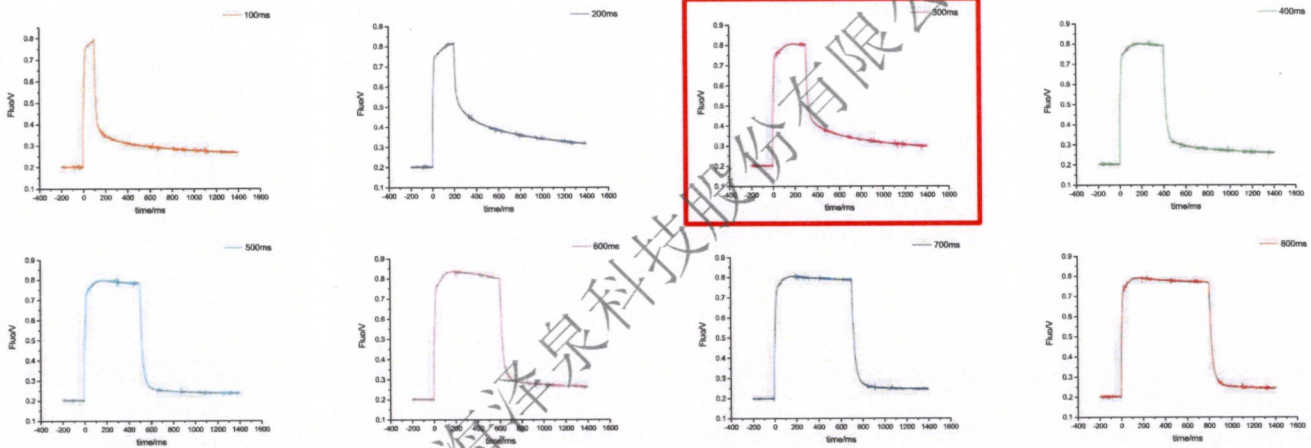
饱和脉冲的理想时间长度应该足够长，但也不能太长，以测到Fm或Fm'，但它不引起非光化学淬灭为宜。

饱和脉冲是中性的，前一个脉冲不会影响后续的脉冲作用效果，不会产生累积效应。

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饱和脉冲(SP)的时间长度属性



原则：脉冲使反应中心全部关闭，但是饱和脉冲期间荧光不下降

2020 Draw by Welkin

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PAM远红光(FR)的功能和属性

PAM远红光 (Far Red, FR)：PSI更有效吸收远红光，可以快速氧化电子传递链

FR是PAM荧光仪内置的稳定光源，具有强度可调节的属性，波长在720-750nm之间。

在光合作用研究中，我们可以利用FR将电子从光合作用的电子传递链中抽走，因此，测量中开启FR可以减少饱和和脉冲后回到 F_0 (或 F_0')所需的时间。

FR还可以有效避免由于PS I周围的环式电子传递和PQ库其他形式非光化学还原而导致的二次 F_0 上升。

使用不同的照光协议

ML→SP: Timing

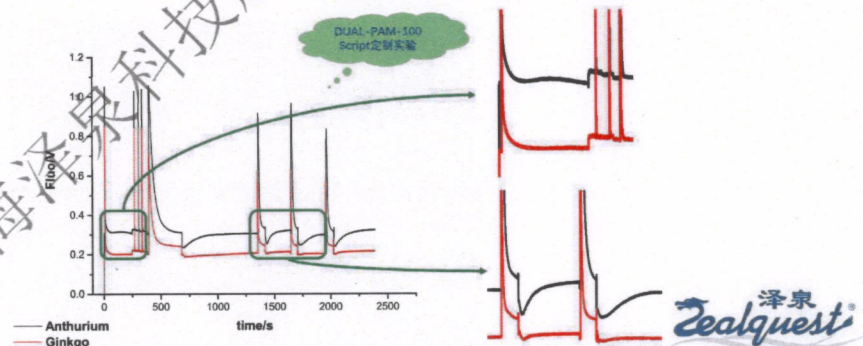
ML+FR →SP: Timing

ML+AL 5Min Off: Timing

ML+AL 50s Off+FR: Timing

ML+AL 50s Off+SP: Timing

ML+AL 50s Off+FR+SP Timing



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PAM Chlorophyll a Fluorescence

Meas Light Int=3, Gain=3, Ft=268 SAT Pulse Int=10, Width=600ms
Act light Int=8, Width=300s Add recovery Y/N

Photosystem Open/Closed
 $Y(NPQ) = F/F_m' - F/F_m$
 $Y(NO) = \frac{F}{F_m}$
 $Y(II) = \Phi_{PSII} = (F_m' - F)/F_m'$
 $AL + Yield$
 $qP = (F_m' - F)/(F_m' - F_0')$
 $qL = qP \cdot \frac{F_0'}{F}$

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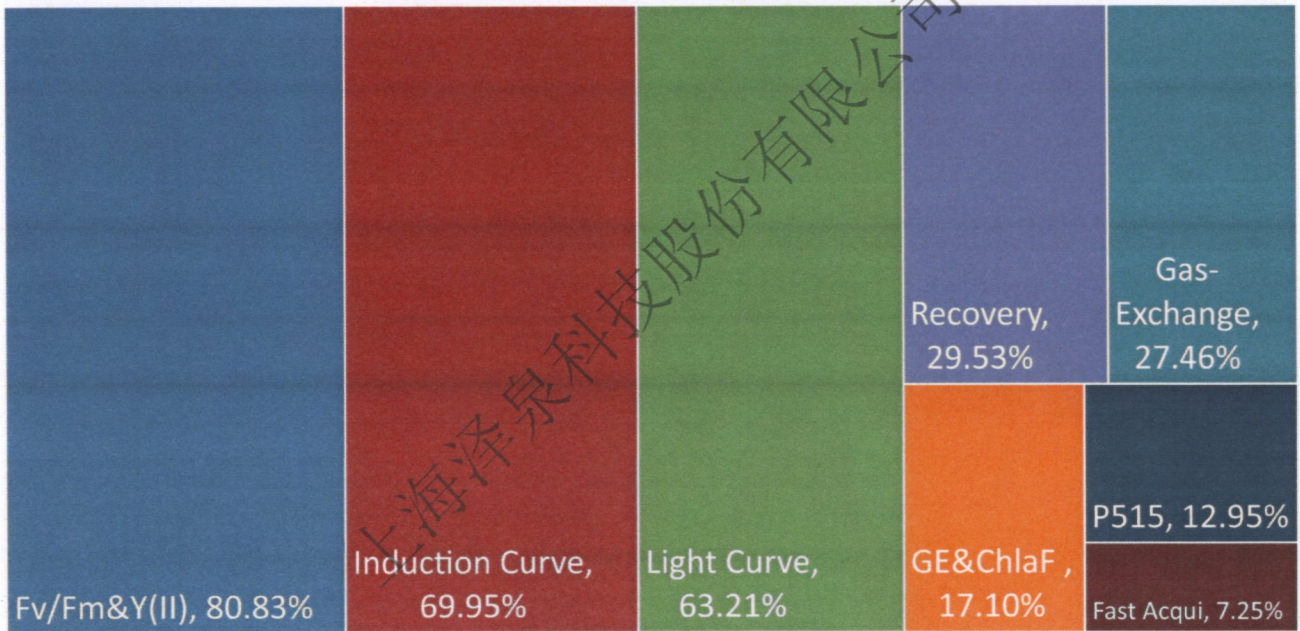
Induction Curve
 Rapid Light Curve
 Dark adapt
 IC+Rec
 Post-illumination Transient Increase in Chlorophyll Fluorescence
 OJIP Fast kinetics
 PSII Acceptor Side
 Donor Side
 OEC: oxygen evolution complex

SP Kinetics
 REG1
 $ETR = ETR_{mPot} \cdot \left(1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}\right) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$
 $ETR_{max} = ETR_{mPot} \cdot \left(\frac{\alpha}{\alpha + \beta}\right) \cdot \left(\frac{\alpha}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}}$
 $I_k = ETR_m / \alpha$
 REG2
 $ETR = ETR_m \cdot \tanh\left(\frac{\alpha \cdot PPFD}{ETR_m}\right)$

LEF: Linear electron flow
 CEF: Cyclic electron flow

@Photosynthesis Engineer

PAM使用情况调查报告



Fv/Fm和ΦPSII



Fv/Fm

ΦPSII或Y(II)

反映光系统II的最大光合活性，
即PSII复合体本身的活性

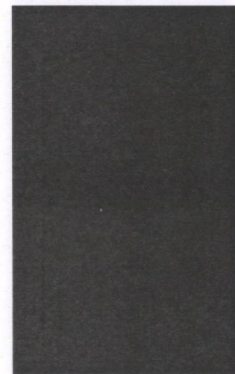
反映光系统II的实际光合活性，
即PSII在稳定光下所反映出来的活性，
线性电子传递相关

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如何进行暗适应？



暗适应叶夹——覆盖式遮挡——暗室——深夜/黎明前

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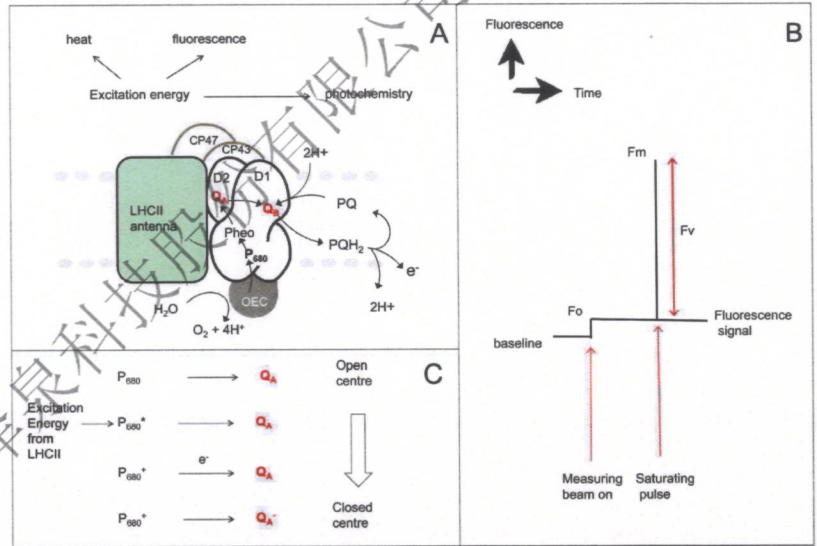
为什么要暗适应?

暗适应: 光系统反应中心完全开放

暗适应使反应中心处于完全开放状态
 只有测量光的情况下, 积分光强非常低
 激发能不足以诱导反应中心持续电荷分离
 产生的激发能主要以荧光的形式重新发射
 测量稳定的基线 $\approx F_0$

饱和脉冲积分光强非常高, 时间很短
 饱和脉冲使所有反应中心全部关闭
 诱导所有反应中心发生电荷分离
 测量光高频, 激发能产生的荧光最大
 测量最大荧光 F_m

可变荧光 $F_v = F_m - F_0$

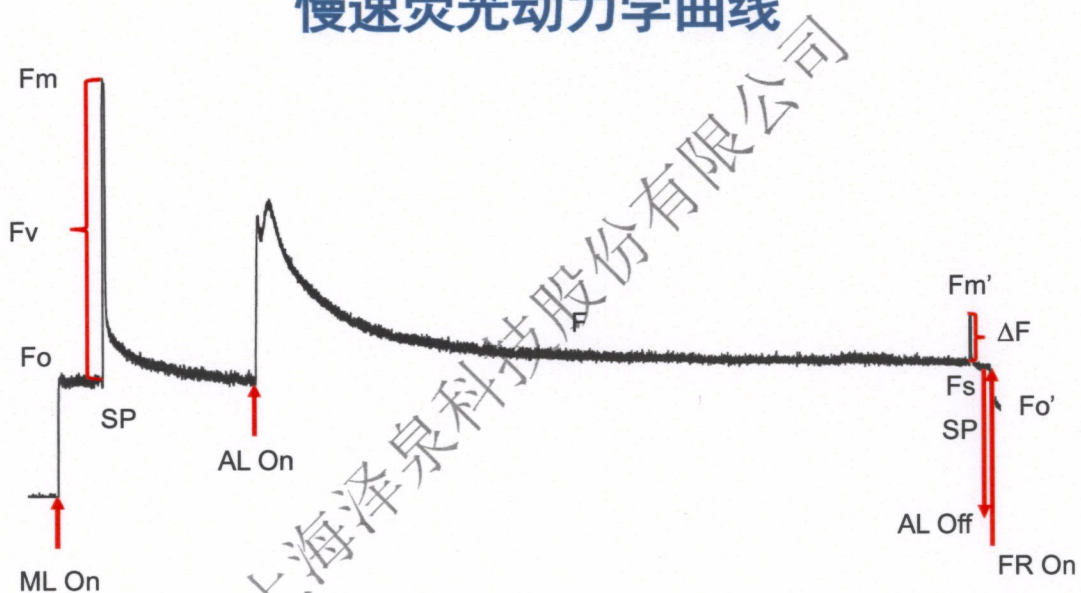


doi:10.1093/jxb/ert208



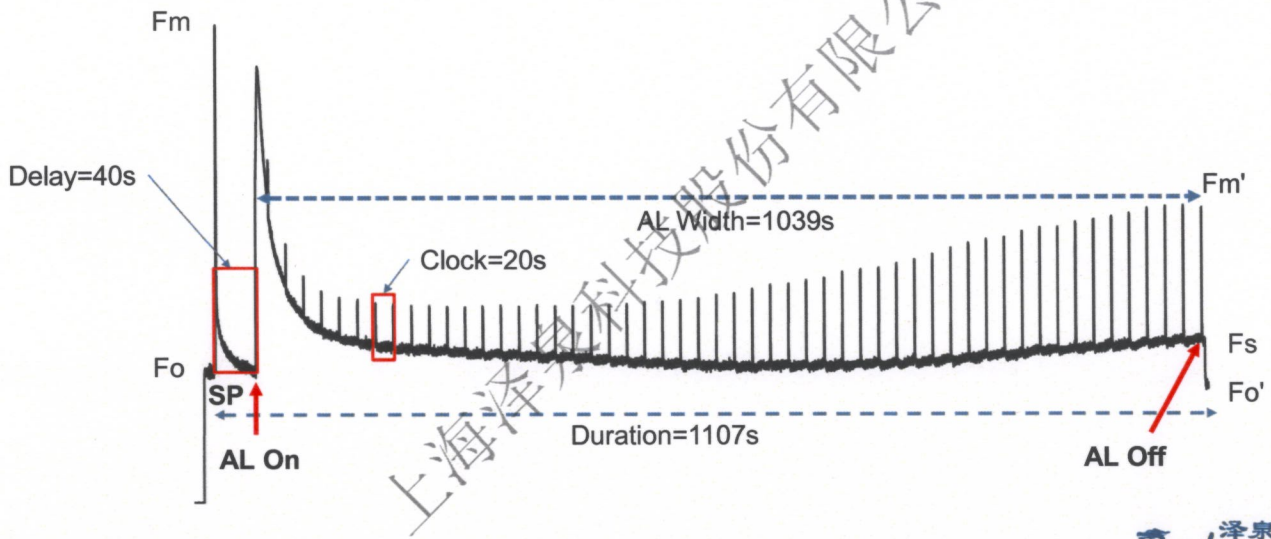
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慢速荧光动力学曲线



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PAM测量慢速荧光诱导动力学曲线及进行淬灭分析



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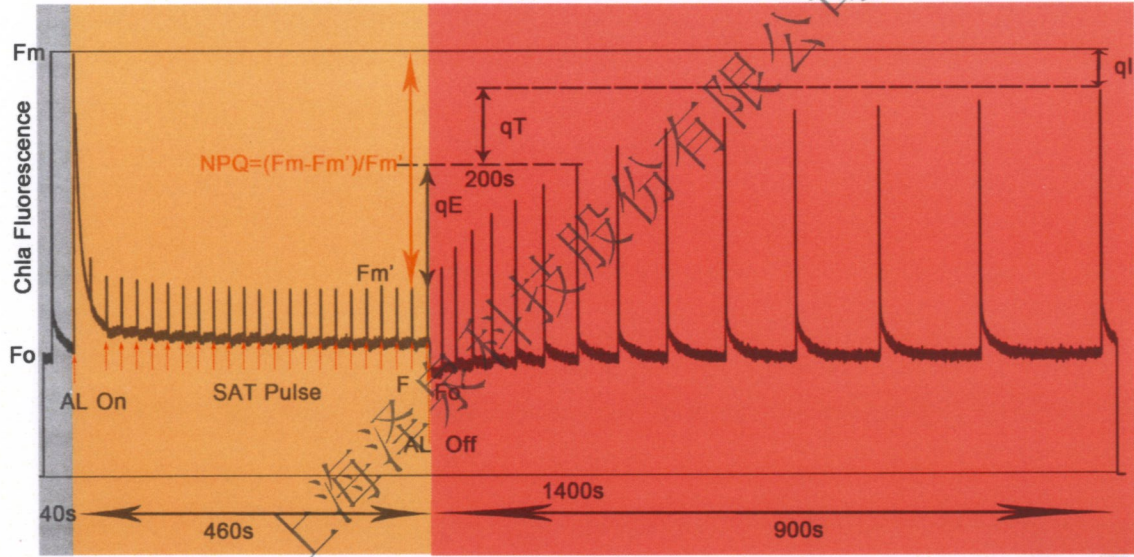
慢速荧光诱导动力学曲线及进行淬灭分析数据范围

参数	计算公式	样品状态	参数范围
F_v/F_m	$F_v/F_m = (F_m - F_o) / F_m$	Dark	[0-1] [0-0.84]
$Y(II)/\Phi PSII$	$Y(II) = (F_m' - F) / F_m'$	light	[0-1] [0-0.84]
$Y(NPQ)$	$Y(NPQ) = F / F_m' - F / F_m$	Dark & Light	[0-1] [0-0.9]
$Y(NO)$	$Y(NO) = F / F_m$	Dark & Light	[0-1] [0-0.9]
NPQ	$NPQ = F_m / F_m' - 1$	Dark & Light	[0-∞] [0-4]
q_P	$q_P = (F_m' - F) / (F_m' - F_o')$	Dark & Light	[0-1] [0-1]
q_L	$q_L = q_P \cdot F_o' / F$	Dark & Light	[0-1] [0-1]
q_N	$q_N = 1 - (F_m' - F_o') / (F_m - F_o)$	Dark & Light	[0-1] [0-0.95]

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PAM测量慢速荧光诱导动力学曲线及暗弛豫

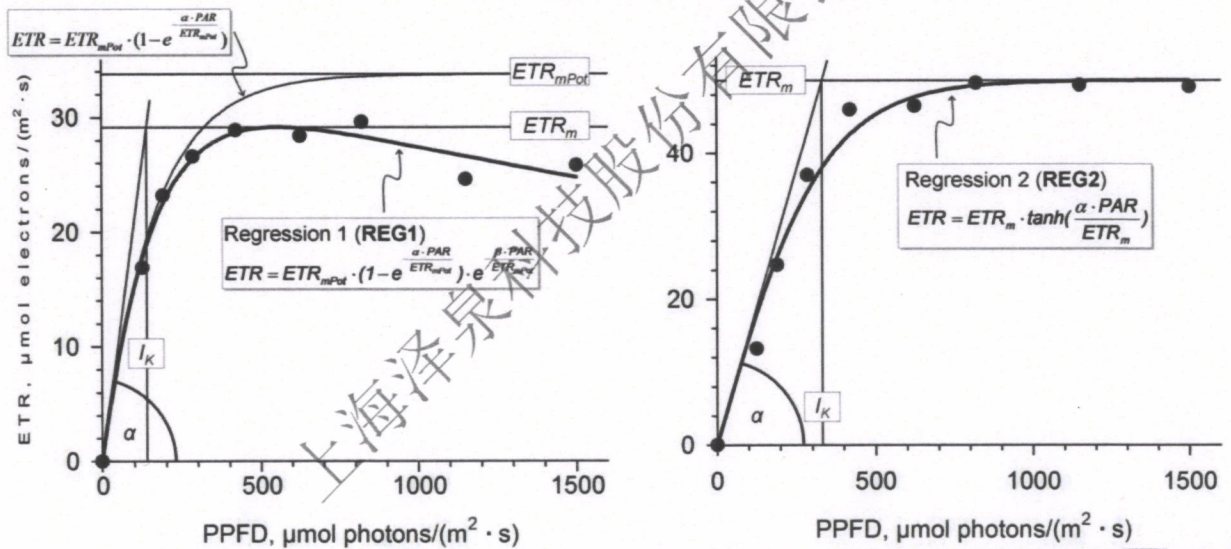


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PAM测量快速光曲线

$ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$, 单位: $\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

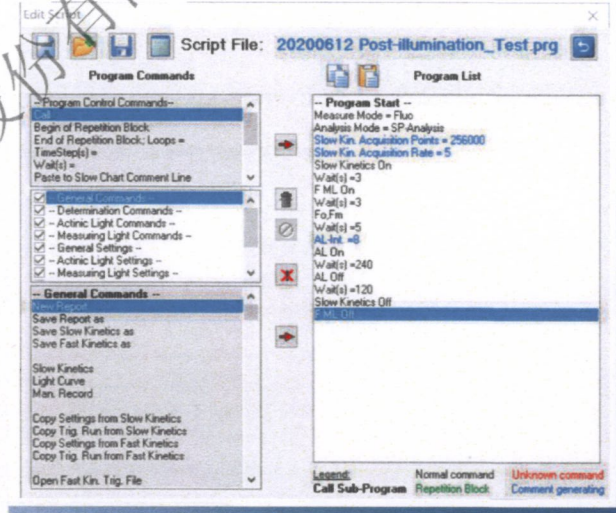
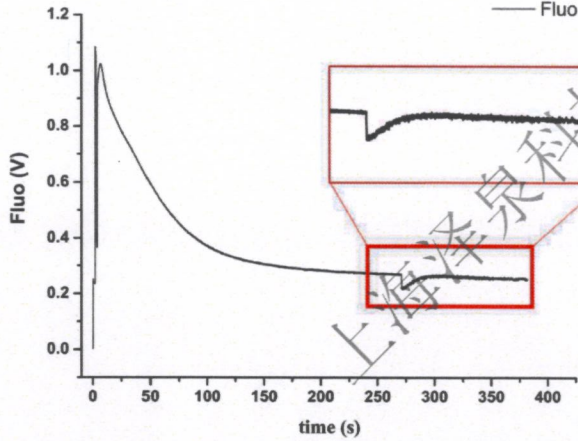


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PAM测量围绕PSI的循环电子传递活性

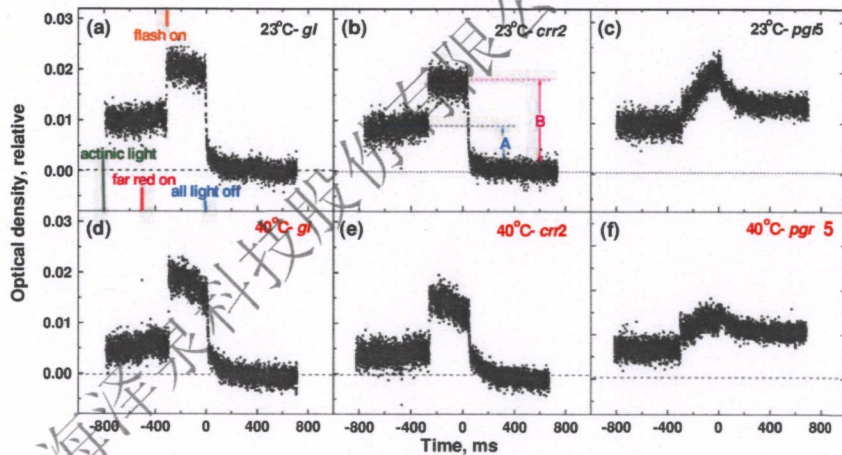
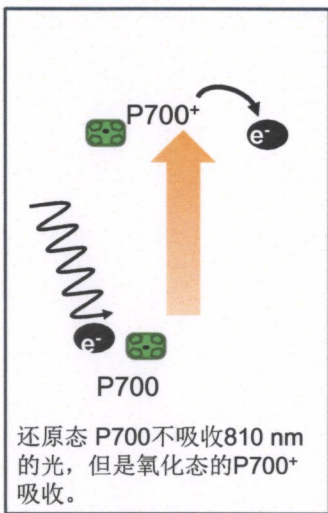
Post-illumination Transient Increase in Chlorophyll Fluorescence

通过PSII荧光反应围绕PSI的循环电子传递活性，衡量NDH-1所介导的环式电子传递速率的技术。



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DUAL-PAM-100测量P700氧化还原



P700 氧化率 = A / B

P700⁺ under actinic light
Max P700⁺ induced by far red and flash



With kind permission from Springer Science+Business Media from Zhang, R. and Sharkey, T. (2009). Photosynthetic electron transport and proton flux under moderate heat stress. Photosynthesis Research, 100: 29-43.

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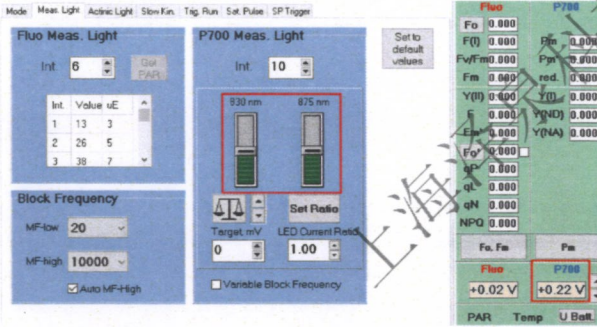
DUAL-PAM-100测量P700的双波长设计提高信号质量

In: Garab G. (ed) *Photosynthesis: Mechanisms and Effects*. Vol. V, pp. 4357-4360. Kluwer Academic Publishers, Dordrecht (1998)

MEASURING P700 ABSORBANCE CHANGES IN THE NEAR INFRARED SPECTRAL REGION WITH A DUAL WAVELENGTH PULSE MODULATION SYSTEM

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Key words: chloroplast, ferredoxin, P700, Photosystem I, transient absorption spectroscopy



3. Result and Discussion

3.1 Improvement of the P700⁺ signal quality by the dual wavelength method

In Fig. 2 an example is presented showing the improvement of the signal quality by the dual wavelength method on light- and chemically induced absorption changes in rapidly stirred suspensions of intact chloroplasts. In case of the dual wavelength measurement (Fig. 2A) a light induced bleaching signal (810-860 nm) caused by reduction of PSI acceptors (3) and a distinct P700 oxidation after addition of 0.5 mM nitrite (open arrow) are clearly visible on top of the background stirring noise (2×10^{-4}). In contrast, when the same experiment was repeated with the standard ED-800-T single wavelength unit (Fig. 2B) the light induced bleaching was obscured by the large stirring noise (2×10^{-3}) and by a slow wavelength-unspecific apparent absorption increase. The P700 oxidation signal caused by addition of nitrite can hardly be assessed against the background of the unspecific change and the stirring noise.

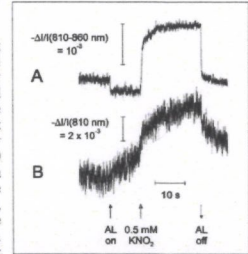
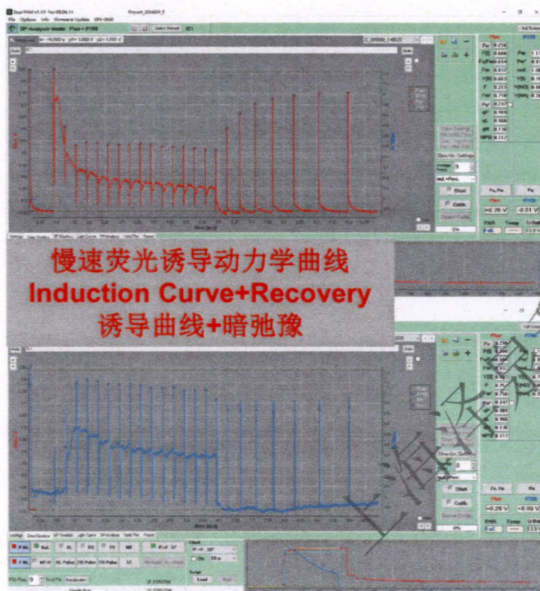


Figure 2. Comparison of dual (A) and single (B) wavelength P700⁺ measurements on suspensions of rapidly stirred intact chloroplasts (60 μg chl/ml). Nitrite was added 10 s after actinic light on (250 μE/m²s).



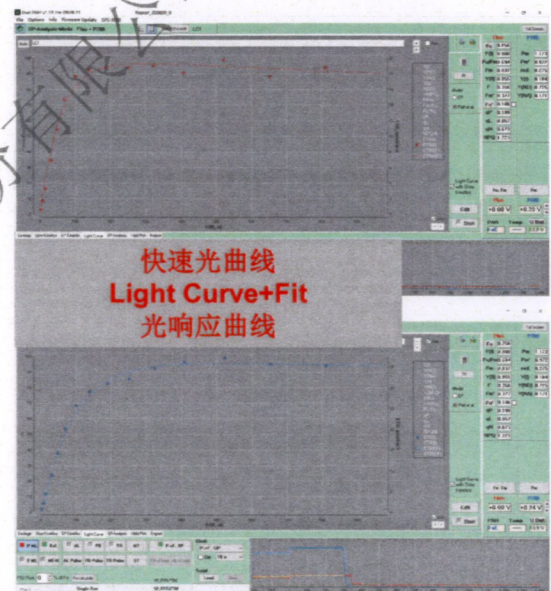
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DUAL-PAM-100实现了PSII和PSI的同步测量



PSII@Fluo

PSI@P700



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DUAL-PAM-100测量P700氧化还原的基本参数

P700: 实时信号，830nm和875nm双波长Balance后的差示信号，在0附近波动

Pm: 远红光适应后打开饱和脉冲测量的最大P700信号

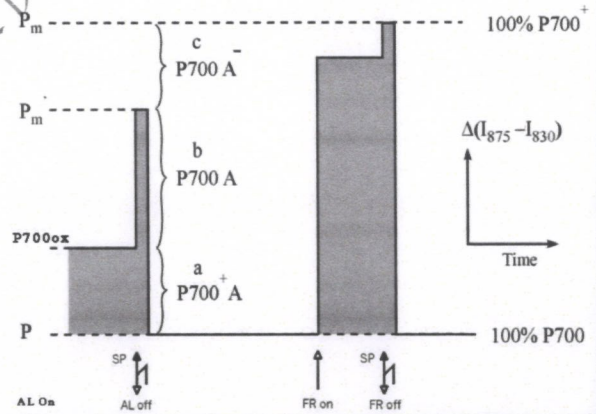
Pm': 给定光照状态下打开饱和脉冲测量的最大P700信号

P700ox: 氧化态P700的信号

Y(ND): $(P700ox - P) / (Pm - P)$: PSI供体侧限制

Y(NA): $(Pm - Pm') / Pm$: PSI受体侧限制

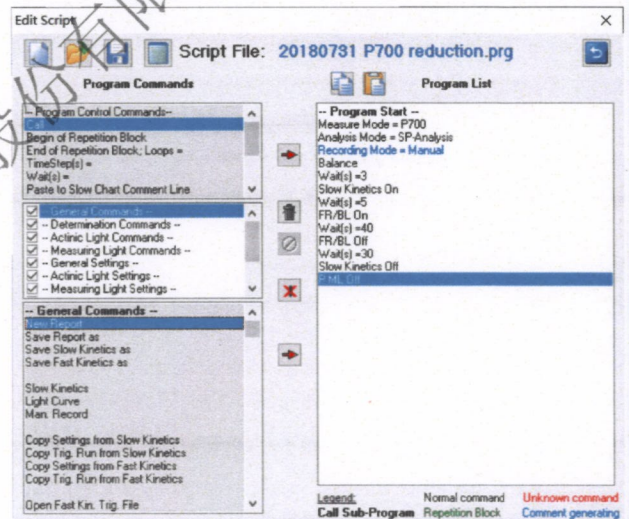
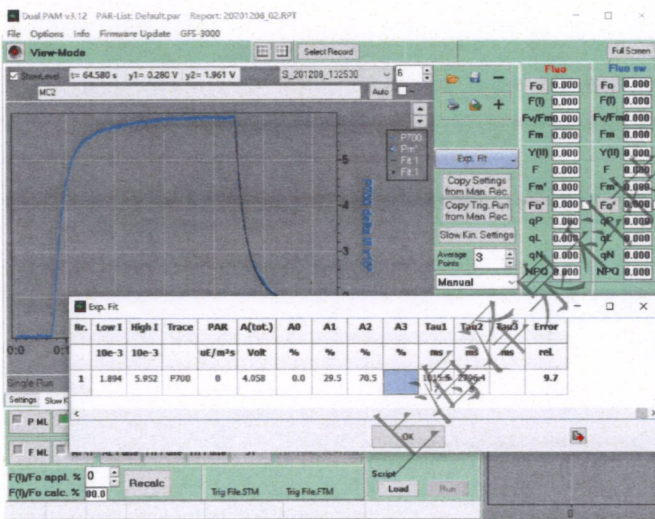
Y(I): $1 - Y(ND) - Y(NA)$: PSI光能转换效率



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DUAL-PAM-100测量P700暗还原反应循环电子传递

Initial rate of dark re-reduction of P700+



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DUAL-PAM-100测量P700还原曲线的面积计算PQ PoolSize

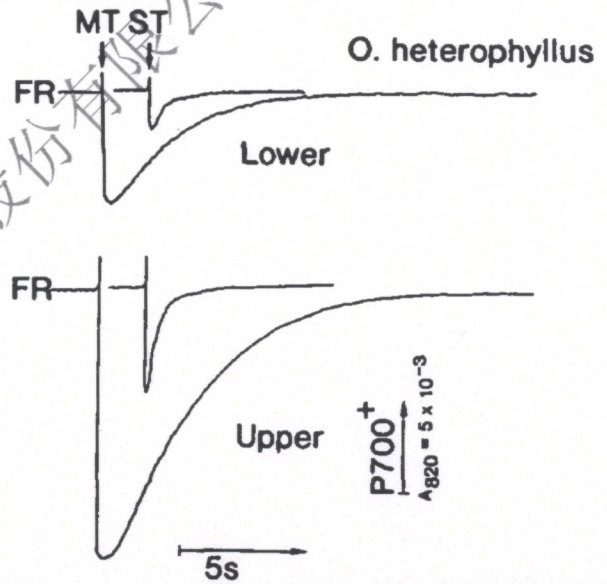
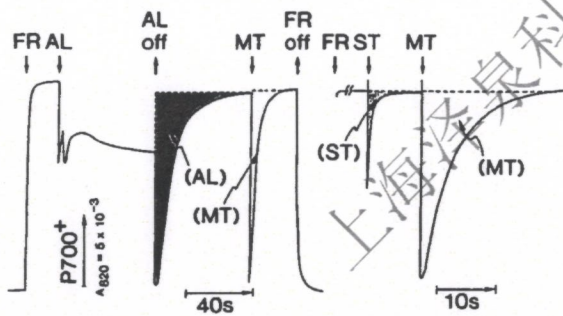
$$PQ \text{ Pool Size} = \frac{Area_{MT}}{Area_{ST}}$$

Plant Cell Physiol. 33(7): 927-932 (1992)
JSPF © 1992

Pool Size of Electrons That Can Be Donated to P700⁺, As Determined in Intact Leaves: Donation to P700⁺ from Stromal Components Via the Intersystem Chain

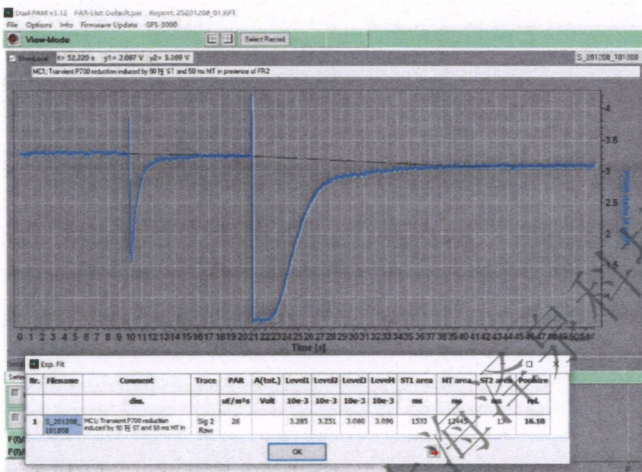
Kozi Asada¹, Ulrich Heber² and Ulrich Schreiber²

¹ The Research Institute for Food Science, Kyoto University, Uji, Kyoto, 611 Japan
² Lehrstuhl für Botanik I, Universität Würzburg, Minster-Dallenbergweg 64, Würzburg, D-8700, Germany



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DUAL-PAM-100测量P700还原曲线的面积测量PQ PoolSize



PQ Pool Size=16.10 (Rel.)

Script File: 20180731PQ pool.prg

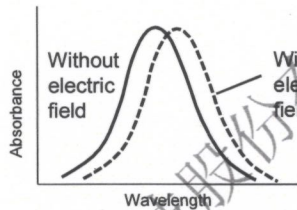
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-- Program Start --
Measure Mode = P700
Analysis Mode = SP-Analysis
Recording Mode = Manual
Balance
Wait(s) = 3
FR/BL On
Wait(s) = 5
Slow Kinetics On
Wait(s) = 3
ST
Wait(s) = 3
MT
Wait(s) = 20
Slow Kinetics Off
FR/BL Off
    
```

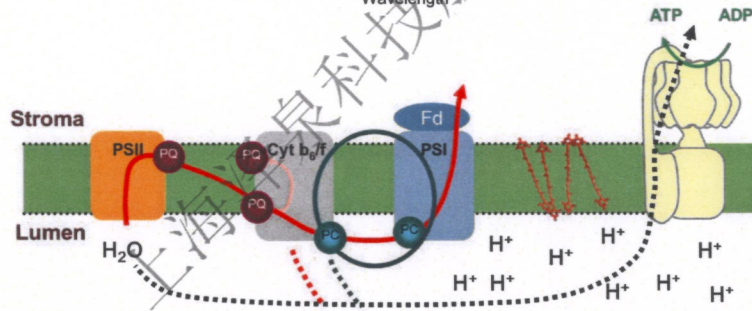
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DUAL-PAM-100测量P515信号暗光暗曲线反应pmf

类囊体膜两侧的质子动力势
(跨膜电位和跨膜质子梯度)
可以通过他们对色素吸收光谱
的影响来测量。



电致变色(ECS)
是色素在变化的电场中表现出的
吸光性能的变化

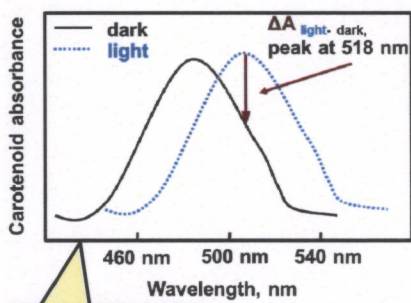


Witt, H.T. (1979). Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods: The central role of the electric field. Biochim. Biophys. Acta Bioenergetics. 505: 355-427.

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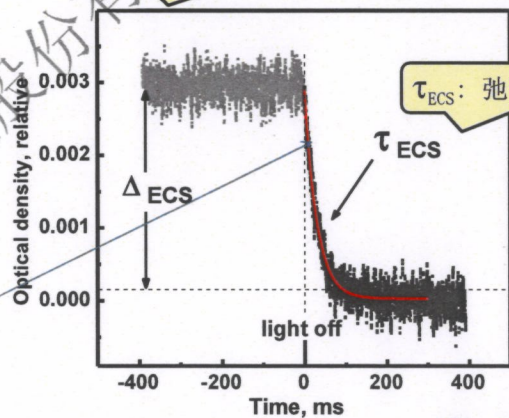
DUAL-PAM-100测量P515信号暗光暗曲线计算pmf



光诱导pmf,
ECS发生

$1/\tau_{ECS}$: 质子导度,与ATP合酶活性成正比。
ECS衰减越快, ATP合酶的活性就越高。

Δ_{ECS} : 光诱导跨类囊体膜的pmf
(质子动力势)



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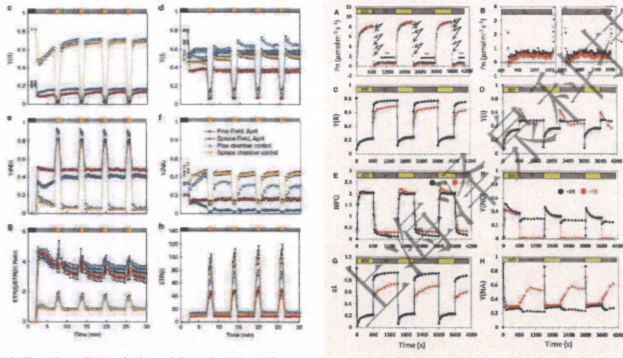
With kind permission from Springer Science+Business Media from Zhang, R. and Sharkey, T. (2009). Photosynthetic electron transport and proton flux under moderate heat stress. Photosynth. Res. 100: 29-43; see also Baker, N.R., Harbinson, J., and Kramer, D.M. (2007). Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 30: 1107-1125.

DUAL-PAM-100在文献中的其他常见应用

测量状态转换 State transitions

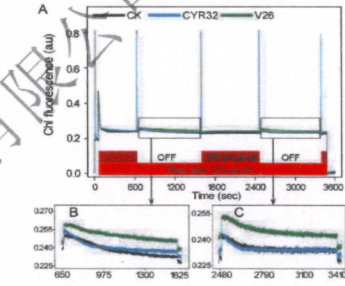
模拟波动光 Fluctuating Light

DUAL-PAM-100 & GFS-3000 联用

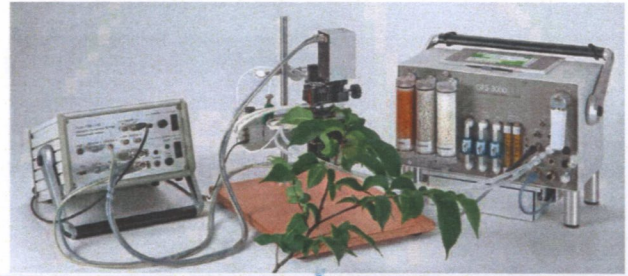


2020-Yang-Nature Communications, doi.org/10.1038/s41467-019-13954-0

2019-Kono-Plant and Cell Physiology, doi:10.1093/pcp/pcz191

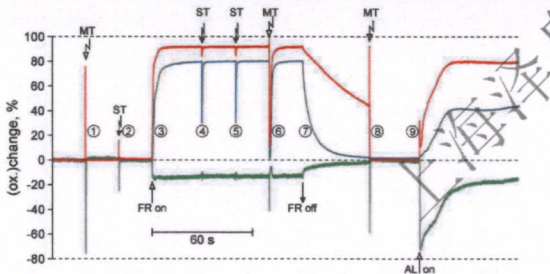
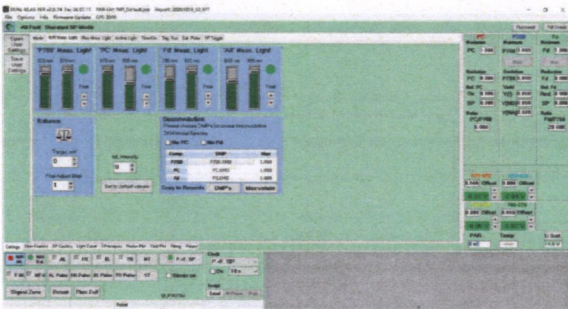


2020-Chen-International Journal of Molecular S



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DUAL-KLAS-NIR近红外光谱仪



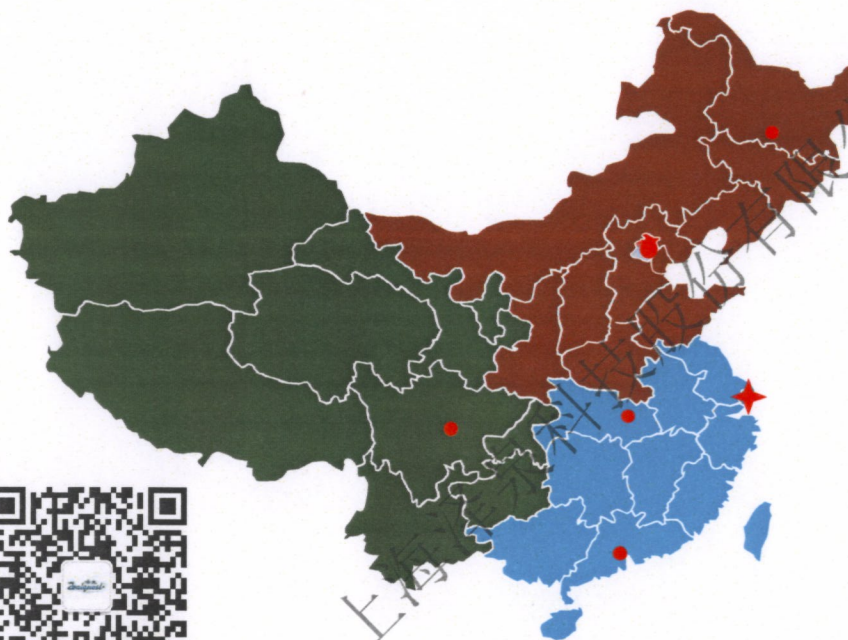
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Relative pool sizes of Fd and PC determined via 100% redox changes

	Fd	P700	PC	Fd/P700(rel.)	PC/P700(rel.)
Ivy	4.3	15.5	7.7	0.28	0.50
Nerium oleander	4.5	17.0	5.3	0.26	0.31
Spinach	2.6	10.2	5.1	0.25	0.50
Brassica	1.96	7.0	3.6	0.28	0.51
Barley	3.1	9.5	7.8	0.37	0.82
Zea mays	1.75	6.4	3.8	0.27	0.59
Amaranthus	2.47	8.9	4.4	0.28	0.49
Taxus	4.0	16.0	9.1	0.25	0.57
Pinus	2.25	9.3	6.1	0.24	0.66
Kalanchoe	2.4	11.4	5.7	0.21	0.50
Sedum	1.6	7.0	2.2	0.23	0.31
Schlumbergera	1.8	6.8	4.8	0.26	0.71
Asplenium (fern)	0.85	4.5	1.82	0.19	0.40

Variation of PC/P700 larger than that of Fd/P700

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- 武汉办事处 18627071855
- 哈尔滨办事处 18745106721

注：上图不是完整中国地图，仅用来演示部分城市大略位置。

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