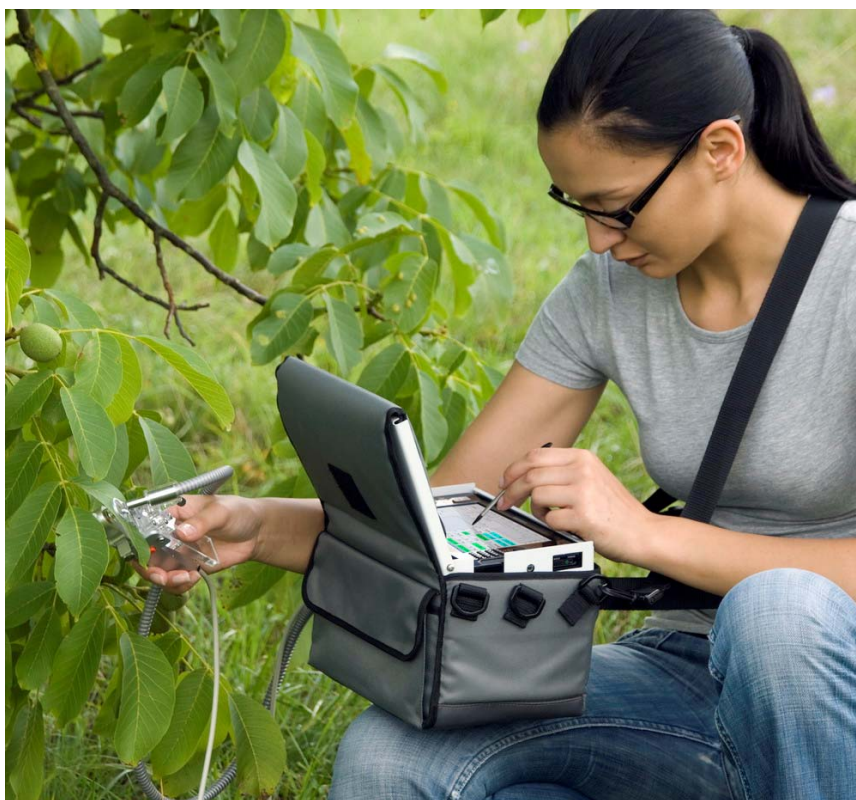


便携式调制叶绿素荧光仪

PAM-2500

操作手册



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1 安全指导

- 1、仪器安装使用前首先阅读安全指导和操作指南
- 2、注意所有的安全警告
- 3、保持仪器清洁，注意防尘，远离水和潮湿的地方。特别是主控单元PAM-2500和叶夹2030-B更应防潮和防尘。只要把敏感部位（如微型光量子探头）用塑料袋包住，即使在极端环境中（如潮湿或有风沙的地方）仪器也可正常操作运行。
- 4、仪器应放置在通风的环境中
- 5、仪器要远离热源
- 6、严格按照操作手册连接电源，**禁止在PAM-2500 开机的情况下连接外接电源！！ 禁止在PAM-2500 开机的情况下通过数据线连接PC或UMPC！！**
- 7、**禁止过度弯曲光导纤维！**
- 8、**禁止将光纤末端对着眼睛，防止灼伤！！**
- 9、光源不用时应当关闭，这样可以延长光源的寿命。
- 10、测量间隙较长时应关闭主机，这样可以省电。**仪器长期放置不用时，应每隔2个月充电一次！**
- 11、每次测量开始前应通过调节“Gain”、测量光强或光纤与样品间的距离使只打开测量光时的荧光**Ft**（即**Fo**）在**300～900** 之间
- 12、只能按厂家推荐方法清洁仪器
- 13、不允许液体或其它东西进入仪器内部
- 14、仪器应由专业人员维修

2 简介

PAM-2500型便携式叶绿素荧光仪是著名的**PAM-2000/PAM-2100**叶绿素荧光仪的升级版。**Walz** 公司于1992 年研制出第一台便携式叶绿素荧光仪**PAM-2000**，它一面世就获得了良好的声誉，并在世界各地得到广泛应用。**PAM-2500** 保留了**PAM-2000/PAM-2100**的所有优点并且在技术上做了很大改进，它的硬件和光学系统都采用了最新的技术。结合最新的超便携个人电脑（UMPC）技术，仪器操作通过最新操作软件**PamWin-3**进行，完全基于**Windows**操作系统，界面友好。

PAM—2500的主要改进如下：

- 利用发光二极管（LED）提供光化光和饱和脉冲，不再使用散热量大的卤素灯
- 光化光为红蓝光源，由发光二极管（LED）提供
- **PAM—2500**的触摸屏使野外操作更为方便
- **PAM—2500**提供单周转饱和闪光和多周转饱和闪光
- 荧光测量的最大时间分辨率为15 μ s，可测荧光诱导曲线的快速上升动力学O-I-D-P相和O-J-I-P相
- 利用强大的UMPC电脑进行操作，完全基于**Windows**操作系统，界面友好
- 60 G硬盘，无限量存储

叶绿素荧光可以通过不同的方法测量，根据不同的应用目的，数据的分析方法也不同。便携式叶绿素荧光仪**PAM-2500** 在荧光测量和数据分析方面具有高度可塑性（flexibility）。但这并不意味着在开始测量前就必须掌握这台多功能仪器的所有特性。实际上，由于软件**PamWin-3**对仪器的控制是“智能化”的，因此操作失误几乎不对仪器本身产生有害影响。另外，也不必当心仪器参数的设置，因为这些都是标准测量设置，即使人为对其进行了改变，也可以随时恢复。因此，即使专业背景很弱的初级用户也很容易掌握测量方法，并且随着测量的进行对专业知识的掌握也会逐渐深入，直至能够进行高级应用。这本手册试图涵盖**PAM- 2500**的所有功能和应用，有些对用户来说可能暂时还用不到，但随着用户对测量结果分析的深入，这些功能可能会成为有用的辅助手段。如果时间允许的话，用户最好逐章阅读本手册，熟悉**PAM-2500** 的所有功能，并且重复列举的实验，以便更好的掌握本仪器。如果用户需要马上就使用本仪器的话，可以阅读第4章第2节“野外屏幕”。

3 PAM-2500 的组件及安装

3.1 基本系统组件

PAM-2500基本系统组件包括：

- PAM-2500主控单元
- 特制的光纤2010-F
- 60° 距离叶夹2010-A
- 电池充电器MINI-PAM/L
- MINI-PAM/AK电缆
- 特制USB数据线PAM-2500/K1
- 荧光标准
- 保险丝
- 背包
- 运输箱2040-T
- 仪器操作及数据采集软件PamWin-3

3.2 基本系统的安装

PAM-2500的安装

- 将PAM-2500主控单元（图3.1 A）放置到一个平面上
- 将光纤的一段通过位于前面板的三孔光纤连接器（图3.1 A）连接到主控单元

注意：不要让任何物品进入光纤连接孔！！

- 将电池充电器连接到仪器前面板的<EXT.DC>插孔，并接通电源（如果内置电池电量充足的话可以不用连充电器）。<Charge>指示灯（图3.1 A）黄灯亮表示正在充电，电池充满后绿灯亮。请注意，外置12 V电池不能给内置电池充电，但是可以通过MINI-PAM/AK电缆给仪器供电。
- 将特制USB数据线连接到计算机
- 按POWER ON键打开仪器。<Status LED>指示灯开始闪烁说明仪器以正常启动可以进行软件连接。当电脑成功连接好仪器后<Status LED>指示灯会一直亮着。如果没有连接电脑而<Status LED>指示灯一直亮着说明PAM-2500发生错误，一般情况下将仪器关掉后等几秒钟后重新开机便可恢复正常。

注意：四个连接插孔（LEAF CLIP、AUX、EXT. DC和USB）不能连错。不能将插头接到错误的插孔里。插头和插孔上的红点指示了连接方向。不要通过拽电缆来拔出插头，应当通过捏住带波纹的金属部位来拔出插头。



图3.1 PAM-2500主控单元前面板

3.3 安装 PamWin-3 软件

- 登录<http://www.walz.com/> 下载最新版PamWin-3（或者使用随机光盘中的PamWin-3），双击软件进行安装。（建议将软件安装到D盘或者E盘等非系统分区）安装完成后会在桌面上出现PamWin-3快捷方式。
- 双击桌面上PamWin-3的快捷方式启动软件。

3.3.1 USB 端口设置

安装 PamWin-3 时，程序会安装 USB 数据线的驱动。第一次启动 PamWin-3 时需要对 USB 端口进行设置，设置方法如下（Windows XP）：

- 首先确保 PAM-2500 已打开，USB 数据线以连接好并且已经成功安装了驱动
- 在桌面右键点击“我的电脑”，选择“属性”
- 点击“硬件”标签
- 点击“设备管理器”
- 选择“端口（Com 和 LPT）”，双击展开
- 选择“USB Serial Port COM#”，双击打开
- 点击“端口设置”
- 点击“Advanced”
- 将“Latency timer”设置为 1ms
- 点击“确定”完成设置

3.3.2 蓝牙无线连接

- 注意：相对 PamWin-3 的<Fast Kinetics>模式来说，蓝牙的数据传输速度太慢，所以当用蓝牙进行无线连接时，<Fast Kinetics>模式不可用。
- 确保 PAM-2500 已打开并且没有通过 USB 数据线进行连接。
- 在桌面或者开始菜单打开“我的蓝牙位置”
- 双击“搜索蓝牙设备”
- 现在设备<PAM 2500 SNR XXXX>会出现在蓝牙列表上(X 代表了 4 位阿拉伯数字)。如果列表中没有 PAM 2500，请调整 PAM2500 和电脑的位置（注意：蓝牙天线在 PAM 2500 的后端顶部）
- 双击<PAM 2500 SNR XXXX>，接着双击<SerialPort on PAM 2500 SNR XXXX >
- 输入“蓝牙安全码”（BlueTooth security code），所有的 PAM 2500 都是输入：**2500**
- 到此，蓝牙连接已连接好

3.4 野外背包

- 1) UMPC 超便携电脑
- 2) 外置电池 000160101314
- 3) 自动充电器 0001X



图 3.2 野外安装 PAM-2500

通过野外背包可以将 PAM-2500 转变成一个功能强大的便携式调制荧光仪，图 3.2 图示了野外背包的组装过程。

3.5 其它组件

3.5.1 特制光纤 2010-F

特制光纤 2010-F 在一个特制插头的帮助下可以连接到 PAM-2500 的前部（仪器右侧）。光纤有三个光学直径不同的末端，在插头的帮助下分别接入对应的插孔中与相应的光学装置相连。在特制的插头内，光纤的三个末端整合到一起与光纤主体相连，光纤主体的光学直径为 6 mm，长 100 cm。

注意：光纤应轻拿轻放。光纤（特别是与主控单元相连的末端部位）不能被过度弯曲，否则可能会导致光纤破损从而影响信号强度。光纤被钢制螺旋和塑料覆盖，在一定程度上避免了过度的弯曲。

3.5.2 60° 距离叶夹 2010-A

“距离叶夹”上有两个隔离环（spacer ring）用来确定光纤前端与叶片间的距离。光纤与叶片呈 60° 角。这样如果光纤前端朝向入射光方向的话，就不会在叶片上产生阴影。样品可以放在透光孔的下部，也可以放在透光孔的上部（图 3.3）。当叶片放在透光孔的上部时，可将叶片抵在叶夹的折叠

部位。当样品为厚叶片、地衣或苔藓时经常放在透光孔下部。

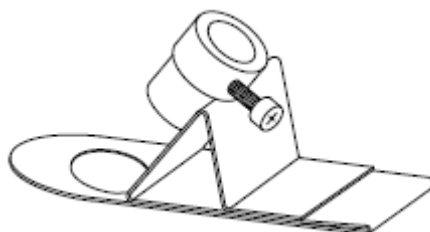


图 3.3 “距离叶夹”，可以调节光纤和叶片之间的距离

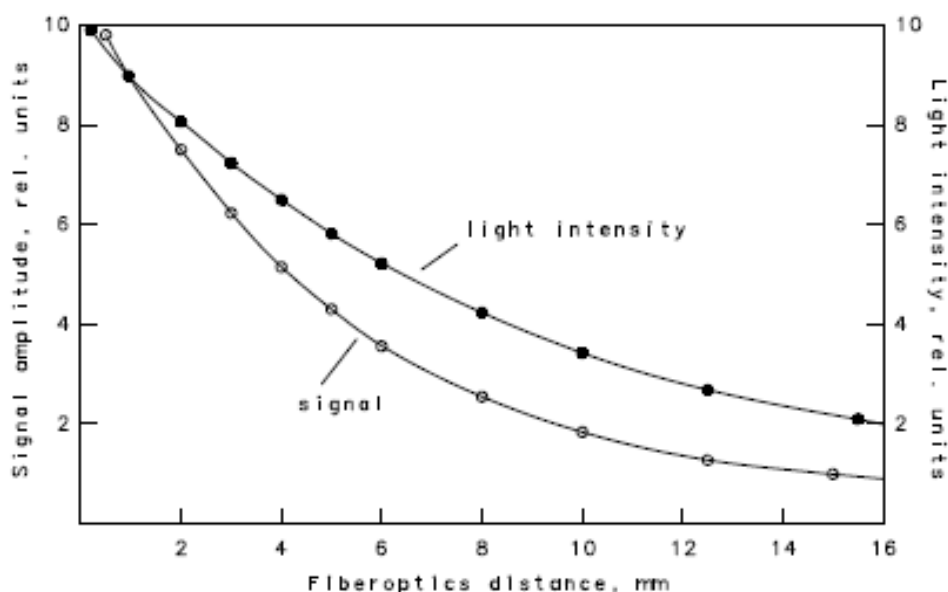


图 3.4 信号强度/光强和样品与光纤间距离的关系

光纤末端与样品间的距离对信号强度和有效光强有影响。由于光纤与叶片呈 60° 角，光纤与叶片间距离的改变不可避免的会产生一系列的有效光强梯度。但这种影响不应被过于夸大，因为在叶片内部由于叶绿体的遮荫作用，存在更大的垂直光强梯度。同时，测量的信号主要由吸收光强最多的叶片部位发出，这些部位被测量光激发的程度最高，光纤接收到的荧光主要由它们发出。图 3.4 描述了信号强度和光强对光纤与样品间距离的依赖性。

3.5.3 叶夹 2030-B

叶夹 2030-B 是 PAM-2100 最重要的附件，在环境光和温度不断变化的野外，它不可或缺。它可以代替标准的“距离叶夹”。其特点是配备了特制的微型光量子/温度探头，每个测量数据都被及时传到 PAM-2500。

叶片被 2030-B 的两个扁平有机玻璃环夹住，该环具有较宽的平面，可以通过垂直调整两个环的距离来适应不同厚度的叶片。光纤与叶片成 60° 角。另外还具有一个 90° 角光纤适配器（2030-B90）可

供选择。光纤与叶片之间的距离可以调整，标准距离由隔离环确定。叶片照光部分由一直径 10 mm 的不锈钢环决定。



图 3.5 插有光纤 2010-F 的 2030-B 叶夹

在叶夹 2030-B 的底部具有连接三角架的螺纹接口。将该支架固定在三角架（如便携式三角架 ST-2101）上有助于对同一植物的长期测量。

在叶夹 2030-B 的手柄部位有一个红色按钮，用于对 PAM-2500 进行遥控。按此按钮就相当于键盘上的回车键。这样可以一手扶着叶片，一手拿着叶夹并遥控操作来测量荧光，特别适合于野外工作。

3.5.3.1 微型光量子探头

叶夹 2030-B 上配有一微型光量子探头，用于测量叶片吸收的光合有效辐射（Photosynthetic Active Radiation, PAR）。在总共 80 mm^2 的测量面积上，探头只占 4 mm^2 ，这基本不影响荧光信号的检测。微型光量子探头的必备光电元件为：

- 一个 1.5 mm 的漫射盘（diffusing disk）；
- 一个直径 0.5 mm 的光纤，用于介导光纤到检测器；
- 一个滤光片组合，用于选择性的测量波长在 380 nm~710 nm 光合有效辐射；
- 一个蓝光增强的硅光电二极管。

除了尺寸小外，探头还保证即使以很小的入射角入射的光线（如日出日落时）也会被检测到。由于探头紧挨着叶片，因此能保证探头测得的 PAR 几乎就是叶片吸收的 PAR。光强单位为 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ 。这样 PAR 就等价于 PPFD（photosynthetic photon flux density，光合有效光量子通量密度）。连接叶夹

2030-B 后，在显示屏的 PAR 参数区会显示测得的 PAR 值。

该探头经过了 LI-COR 光量子探头的校准。校正的稳定性依赖于探头表面是否清洁。建议用标准光量子探头定期校准。任何偏差均可在 PamWin-3 程序中输入校正因子来校正（见 4.3.1.1）。当校正因子（由初始值 1.000）明显升高时，表明探头表面不清洁，需用酒精棉球轻轻擦拭。

需要指出的是，在许多应用中，荧光参数的合理解释需要获得荧光测量点的 PAR 值。因此带光量子探头的叶夹 2030-B 可以被认为是 PAM-2500 最重要的附件。测得的 PAR 值会自动存入报告文件中。

3.5.3.2 热电偶

叶夹 2030-B 带有一个 NiCr-Ni 热电偶，用于测量叶片温度。其末端为一圆环，可以轻轻接触叶片反面。这样既能有效测量温度，又防止了阳光直射。参照电偶位于电路板上，靠近热电压计放大器，密封于叶夹底部。热电压与温度几乎完全呈线性关系。当温度降低时， $\Delta V/^\circ\text{C}$ 也稍有降低。校准是在 25°C 进行的。在 0°C 和 -15°C ，偏差分别为 0.5°C 和 0.8°C 。在 PamWin-3 程序中可以输入零偏移值（见 4.3.1.1），其精度为 0.3°C 。当连接叶夹 2030-B 时，测得的温度显示在显示屏的 Tmp 参数区。温度精度为 0.3°C 。测得的温度也会自动存入报告文件中。

3.5.4 微型光量子/温度探头 2060-M

微型光量子/温度探头 2060-M 可以测量 PAR 和温度，这种功能与叶夹 2030-B 相同，只不过 2060-M 不是固定在叶夹上的。此装置主要是为非叶形样品如地衣、苔藓等设计的。这两个微型探头可以放在荧光测量部位，但不影响荧光测量。它们也可以装在“距离叶夹”（“Distance Clip”）上。

3.5.5 暗适应叶夹 DLC-8



图 3.6 暗适应叶夹

暗适应叶夹 DLC-8 重 4 g, 因此可以直接夹到多数叶子上, 而不会伤害到叶片。在叶夹上装有一个滑片, 可以在暗适应时阻止光线照射。实际测量时, 打开滑片, 使叶片只受到来自光纤的光, 而不受环境光干扰。只有经过合理的暗适应, 才能得到较好的最大量子产量 F_v/F_m 和暗-光诱导动力学曲线。

在使用暗适应叶夹 DLC-8 时, 光纤与叶片间呈直角, 叶片与光纤末端间的距离约为 7mm。这样就导致荧光信号比使用叶夹 2030-B 时 (光线入射角 60°) 高的多。为了避免检测器饱和, 需降低测量光强度或 Gain (增益)。

当滑片仍关闭时打开测量光, 也会有部分 F_t 信号。这些信号是由于部分测量光被滑片反射而干扰检测器的结果。当打开滑片时这种干扰就几乎没有了, 所以不影响正常测量。

4 使用 PAM-2500

4.1 PamWin-3 帮助文档

PamWin-3 的每步操作都提供一个在线帮助文档。通过下列途径打开帮助文档：

- 将鼠标移动到想要得到帮助的地方，稍等片刻即可出现帮助文本提示
- 将鼠标移动到想要得到帮助的地方，按 F1 弹出帮助文档

4.2 野外屏幕（Field Screen）

PamWin-3 的野外屏幕（Field Screen）适用于 PAM-2500 的野外操作。在野外通过 UMPC 的触摸屏对<Field Screen>进行操作。<Field Screen>分为三个区，分别为：字母数字区（alphanumeric fields）、程序和脚本控制区（program and script control）和监测图形显示区（monitoring graphs）（图 4.1）。

字母数字区（alphanumeric fields）显示了饱和脉冲分析的重要信息，同时也包含一个 **Zoom In** 按钮，这个按钮可以切换字母数字区（alphanumeric fields）为全屏显示。

<Field Screen>显示了两个不同类型的图形（图 4.1）左边监测图形显示了慢速荧光变化曲线，同时也显示了 F_m 和 F_m' 。通常来讲进行饱和脉冲分析时只有 F_m 或 F_m' 在该区显示时，饱和脉冲的数据才会记录到报告文件中。如果监测图形（monitoring graphs）被停止后，通过 **SAT** 键执行的饱和脉冲记录的数据不会记录到报告文件中，可以通过点击 **Report** 按钮查看报告文件。

PamWin-3 启动后，慢速动力学监测图形会自动启动。点击 **Fv/Fm** 按钮（见下文）后将重启慢速动力学监测图形，也可以通过点击 **Stop/Start** 来重启慢速动力学监测图形。测定 F_v/F_m 时程序会自动调整 Y 轴坐标从而使图形完全显示出来。荧光信号值可以从 Y 轴读取。在每个图形中，程序都会用黄色和红色的虚线将 F_o 和 F_m 的值分别标示出来。

右侧监测图形显示了当前饱和脉冲的荧光曲线（1.6S 钟长），除了 F_o 和 F_m 外，程序用黑色虚线将 F_m' 的值标示出来。

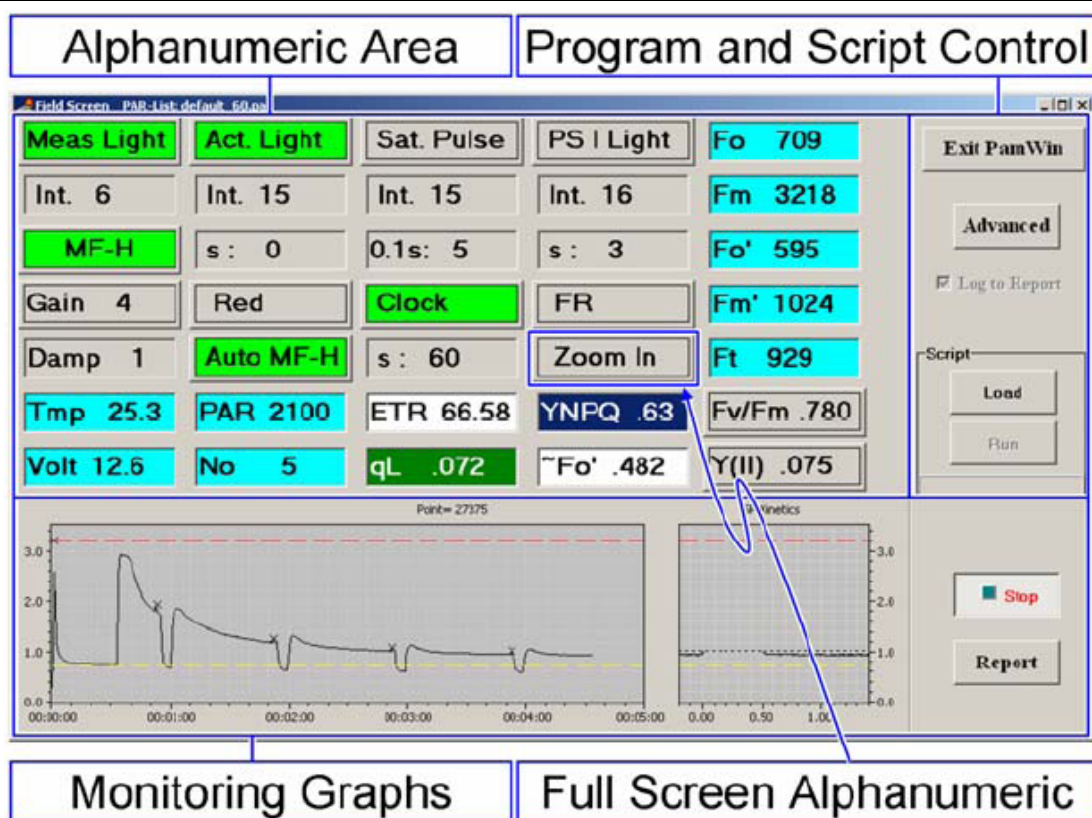


图 4.1 野外屏幕总览

程序和脚本控制区包括退出程序的按钮 **Exit PamWin**、高级设置按钮 **Advanced** 和载入自定义脚本的按钮 **Load** 和 **Run**。

4.2.1 字母数字区（alphanumeric fields）

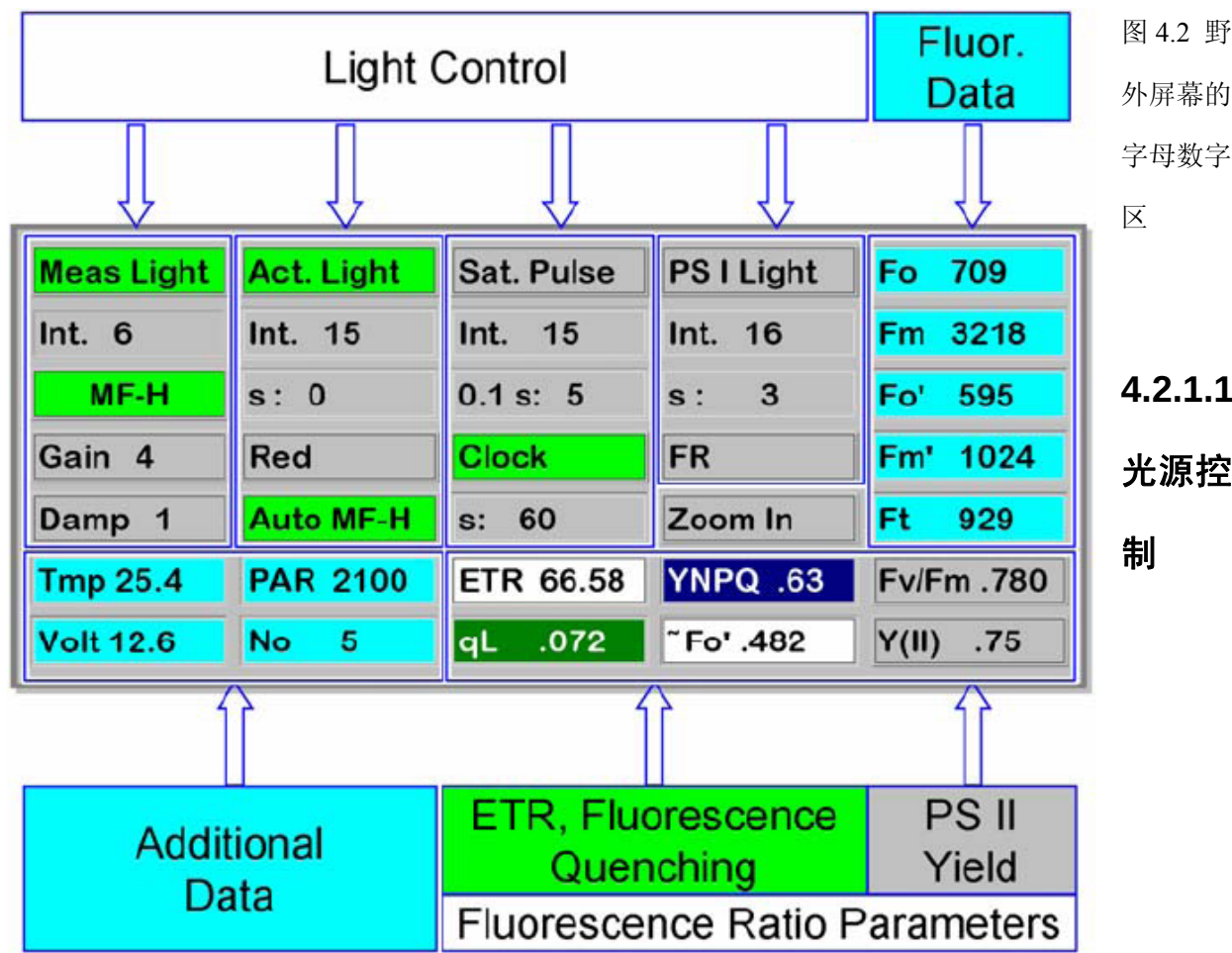
字母数字区可以分为 4 组：a) 光源控制；b) 主要荧光数据；c) 荧光比值参数；d) 其它数据，光强和温度等（图 4.2）。

组一：包括状态按钮和调节按钮。按钮状态的变化可以开/关某个功能或者调节某个值。可以通过鼠标左键的点击来改变按钮的状态，当某个按钮的背景是绿色的时候表明当前按钮执行的功能处于激活状态。控制光源颜色的按钮以文字形式将当前光源的颜色显示出来。

调节按钮的值可以通过三种方式调整：1) 在按钮上点击鼠标左键后用滚轮进行调整；2) 在按钮上双击鼠标左键后输入数值；3) 用 UMPC 操作时，轻点按钮然后用上下箭头进行调整，方向键的下箭头为减，按住 shift，再按方向键的上箭头为加，或者直接按加号也可增加数值。

组二，组三和组四显示了当前测量的相关信息。当用鼠标点击 Fv/Fm 或者 Y(II)按钮时都会执行一

个饱和脉冲（见 4.2.1.3）。



- Meas Light

该按钮用于开/关测量光
-
- Int.

调节测量光强度，共有 20 档可选。在 PamWin-3 的高级设置中提供了每档测量光的光强。可以通过调整测量光的强度调整荧光信号的高低。
-
- MF-H

切换调节测量光的频率。该按钮为灰色背景时说明测量光处于低频（比如测 Fo 时），当按钮为绿色背景时说明测量光处于高频。
-
- Gain

调整电子信号的增益，共有 10 档。当增益为 10 时，信号放大倍数是增益为 1 时的 7 倍。可以通过调整增益的大小调整荧光信号的高低，升高增益，不仅放大荧光信号，**噪音也被放大**。
-
- Damp

阻尼设置，共有 9 个梯度分别为：关闭阻尼，梯度 1（t(1/2)=10 μS）到梯度 8（t(1/2)=4 mS）。阻尼限制了信号变化的最大速率，任何比设置的时间常数快的信号波动（噪音）都会被抑制。注意：在低频测量光时，响应时间由当前采样速度决定，因此比上面列出的值都要低。

Act. Light 打开/关闭光化光。

Int. 调节光化光的强度。

S: 设置光化光持续时间，单位为 S，当设为 0 时需要手动开/关光化光。

Red (Blue) 切换光化光为红光或蓝光。

Auto MF-H 当<Auto MF-H>被激活后，打开光化光时测量光频率自动升高，这样可以降低信噪比。

强烈建议一直将此按钮处于激活状态。不论<Auto MF-H>是否激活，当执行饱和脉冲时测量光的频率都会自动上升到 100 KHZ。

Sat. Pulse 点击该按钮将执行一个饱和脉冲。得到的数据显示在字母数字区 (alphanumeric fields) (图 4.2) 但不可能会被记录到报告文件。

Int. 调节饱和脉冲光的强度。共 20 个梯度可调，梯度 1 和梯度 20 对应的光强分别是 910 和 16500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 。光强梯度和强度之间是半线性相关的。

0.1S: 调节饱和脉冲的持续时间。

Clock 激活后程序将每隔一定时间连续执行饱和脉冲。

S: 连续执行饱和脉冲时，设置两个饱和脉冲之间的间隔时间。

PS I Light 点击后打开第二个光源。如果当前光化光是红光，<PS I Light>可能是远红光 (740 nm) 或者是蓝光 (460 nm)。远红光主要激发植物和大多数真核藻类的 PS I，而蓝光主要用来激发蓝藻的 PS I。当然，光化光设置为红光时，<PS I Light>设成蓝光可以作为植物和真核藻类的第二种光化光光源。

Int. <PS I Light>的强度。由于只有一小部分的远红光被 PS I 吸收，并且不同植物对远红光的吸收率存在很多差别，所有报告文件里没有记录远红光的光强。

S: <PS I Light>的持续时间，设为 0 时需要手动开/关<PS I Light>。

FR (Blue) 当光化光设为红光时，这个按钮用来切换第二种光源<PS I Light>的类型（远红光或者红光）。

4.2.1.2 荧光数据

样品暗适应后执行饱和脉冲记录了两个荧光数据（图 4.2 Fluor.Data）：

Fo 最小荧光产量

Fm 最大荧光产量

照光后样品执行饱和脉冲记录了三个荧光数据：

Fo' 光下最小荧光产量。关闭光化光后打开远红光可以测量 Fo'。可以在<Advanced Level>和<General Settings>中激活 Fo'模式。Fo'模式未激活时，Fo'显示的值为 0。

Fm' 光下最大荧光产量。

Ft 在执行饱和脉冲前测量的实时荧光值 F。很多参数的计算需要用到此数值（见第 5 章）。

4.2.1.3 荧光比值参数

两个反映 PS II 光能利用效率的参数计算如下（见 4.2 PS II Yield）：

Fv/Fm = (Fm-Fo)/Fm; PS II 的最大光合量子产量，反映了样品的光合潜能。

Y(II) = (Fm'-F)/Fm'; PS II 的实际光合量子产量。

其它的荧光比例参数以反背景色显示出来（图 4.2: ETR, Fluorescence Quenching）。所有参数的计算都参照第五章：缩写和公式。

一共有 8 个参数可用，但是每次只能显示 4 个。点击 4 个数据显示面板可以选择要显示的参数。注意这些参数的计算公式（见第 5 章），如果要用到 Fo'而没有测 Fo'时就需要通过 Fo, Fm 和 Fm'来推导 Fo'（Oxborough and Baker 1997, 见第 5 章）。

这 8 个参数分别是：

ETR 通过 PS II 的电子传递速率

~FO' 光下最小荧光

qL 光化学淬灭系数（基于“湖泊模型”）

Y(NO) 非调节性能量耗散的量子产量

Y(NPQ) 调节性能量耗散的量子产量

NPQ 非光化学淬灭系数

qN 非光化学淬灭吸收。qN 和 NPQ 都是反映了非光化学淬灭，只是计算公式不同。

qP 光化学淬灭系数（基于“沼泽模型”）

4.2.1.4 其它数据

Tmp 当连接 2030-B 时显示了叶片下表面的温度，单位：℃。

PAR 当连接 2030-B 时显示的是叶夹测量的光合有效辐射，单位： $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 。当未连接 2030-B 时显示的是从内置光强列表读取的光化光的光强和 PS I 的光强。在 PamWin-3 的高级设置的<General Settings>窗口可以关闭从内置光强列表读取光强的功能。

Volt 电池电压，电池完全充满电时电池电压可以达到 13.7 V。当电池电压低于 10.5 V 时，PAM-2500 仍可以工作，但是此时得到的信息不可靠，特别是进行饱和脉冲时（所需电流太高）。

注意：当电池电压低于 9.4 V 时 PAM-2500 必须关机！！

注意：当电池电压低于 11.8 V 时 PAM-2500 无法开机！！

NO 显示了当前实验饱和脉冲的总数，点击<Stop/Start>按钮或点击 Fv/Fm 后程序将重新计数。

4.2.2 用“野外屏幕”进行第一次测量

本小结介绍了如何利用“野外屏幕”进行叶绿素荧光的基础测量。

首先，确保测量光已打开，此时在光纤末端能看到微弱的红光。不夹叶片或者其它能发荧光的物品时 Ft 的值应该接近 0。当夹上叶片后，仪器便会检测到荧光信号，荧光信号的强弱和光纤距样品间的距离直接相关，因此为了保证每次测量的可比性，样品到光纤的距离应该保持一致。距离叶夹 2010-A 和叶夹 2030-B 都可以保证光纤到样品距离的一致性。

比较不同光强下的荧光产量可以得到一些相关信息，因此 PAM-2500 提供了不同的光源。点击 **Act.**

Light 打开光化光，叶片介绍相对较强的光照射，同时，Ft 迅速上升然后慢慢下降，这就是“Kautsky-effect”。再次点击 Act. Light 关闭光化光，Ft 下降。

当 PS I Light 选为远红光（FR）时，关闭光化光后点击 PS I Light 打开远红光一段时间（一般为 3S），在远红光的照射下 Ft 迅速下降，逐渐接近打开光化光之前的水平。远红光优先激发 PS I，在没有光化光的情况下，PS I 接收远红光后迅速从供体侧获得电子，从而使 PS II 的反应中心迅速打开。

样品暗适应后所有的 PS II 反应中心都处于开放状态时荧光产量最低记作 Fo，此时打开饱和脉冲所有的 PS II 反应中心都关闭，荧光产量达到最大记作 Fm，这一过程可以通过点击 Fv/Fm 执行。Fv/Fm 通过 Fo 和 Fm 计算， $Fv/Fm = (Fm - Fo) / Fm$ 。Fv/Fm 反映了植物的最大光合潜能，对大多数高等植物来说，一片健康的叶片经过充分的暗适应后，Fv/Fm 值大约在 0.8 左右。

点击 Y(II) 同样执行饱和脉冲，测定的值显示在 Y(II) 区。当样品经过暗适应后，测得的 Y(II) 等同于 Fv/Fm。打开光化光后测得的 Y(II) 是当前照光状态下 PS II 的实际光合量子产量。

点击 Clock 后会间隔一定时间连续执行饱和脉冲，相邻两个饱和脉冲的时间通过 Clock 按钮下的 S: 按钮来设定。

执行饱和脉冲时测定的数据会自动存储到报告文件中，可以通过点击 Advanced 按钮并选中 <report> 标签查看。

4.3 PamWin-3 高级设置

4.3.1 常规设置 (General Settings)

图 4.3 显示的是<General Settings>窗口, 和<Field Screen>窗口功能相同的按钮或者显示信息相同的区域以灰色标示出来, 在下文中不再介绍 (注意: 8 选 4 的参数区 (见 4.2.1.3) 显示在侧栏)。

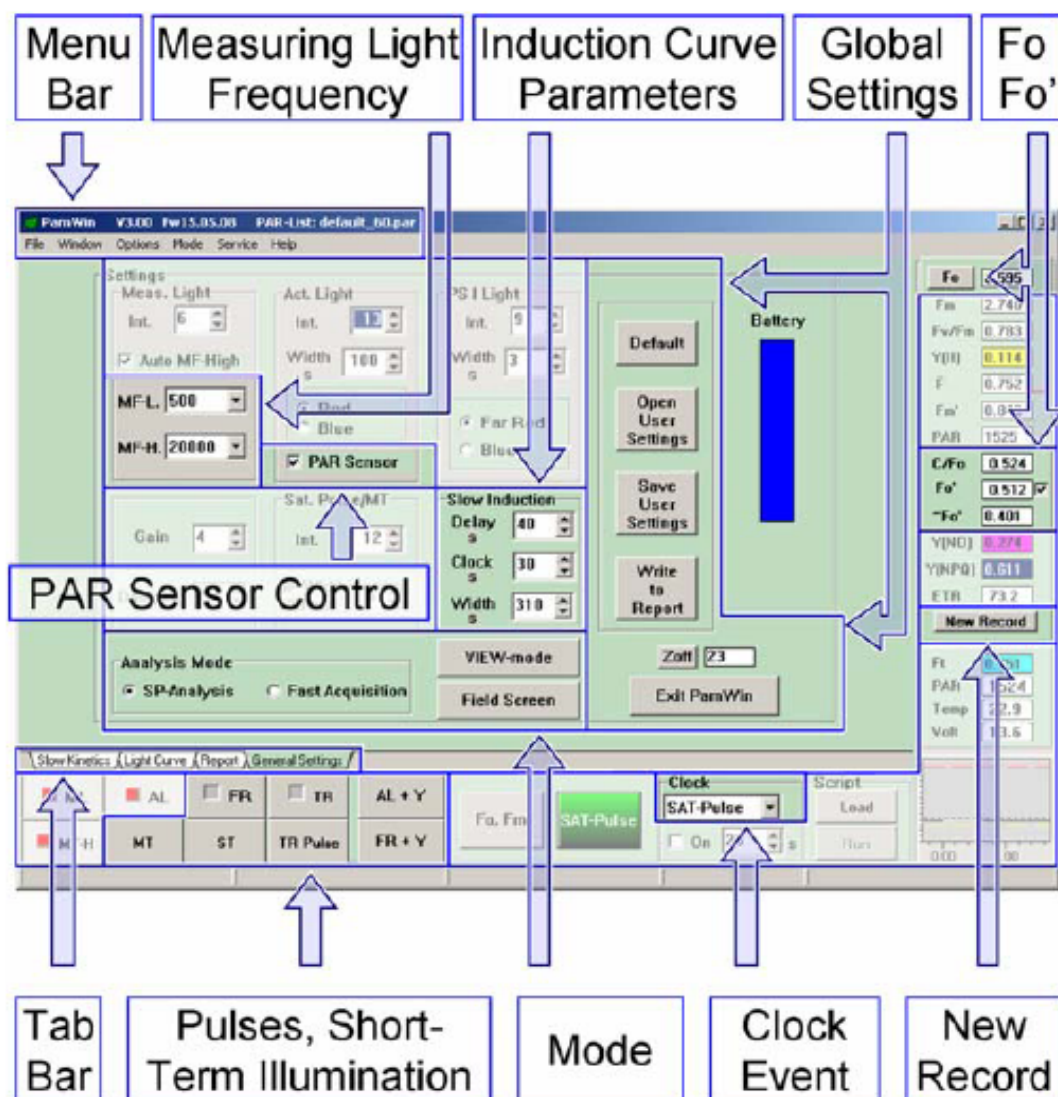


图 4.3 常规设置窗

4.3.1.1 菜单栏 (Menu Bar)

<General Settings>窗口的菜单栏包括 6 个菜单 (图 4.3)。

File

从 File 菜单可以导入，执行 PamWin-3 的脚本程序，也可以进行打印设置，退出程序等操作。

Window

<Window>菜单等效于<Tab Bar>（图 4.3）。<Window>菜单链接到<SP Analysis>模式中的 4 个窗口。
在<Fast Acquisition>模式中，<Window>菜单链接<Fast Acquisition>模式中的 3 个窗口。

Options

L Curve Fit Parameters

点击<Light Curve Fit Parameters>打开光响应曲线拟合参数窗口，并列出了 4 个从拟合公式中得到参数（见 4.4.2 光响应曲线和 5.5 理论知识），分别是：

Fv/Fm x ETR factor/2

暗适应后样品，由 1 个饱和脉冲闪光引起的最大电子传递的产量 electrons/photons

alpha

光响应曲线的初始斜率，相当于最大光合效率 electrons/photons

ETRmax

最大电子传递速率 $\mu\text{mol electrons}/(\text{m}^2 \cdot \text{s})$

Ik

最小饱和光强（半饱和光强） $\mu\text{mol electrons}/(\text{m}^2 \cdot \text{s})$

Light Calibration

输入校正系数对连接到 PAM-2500 的光量子传感器进行校正（校正系数的范围为：0.2~0.5）。连接 2030-B 时默认的校正系数是 1.000。

Light Offset

对光量子传感器进行调零，将光量子传感器放到完全黑暗的环境中点击 **Auto Zero** 进行自动调零，当然也可以通过手动输入数字进行调零。

Temperature Offset

对外置温度传感器进行调零。

ETR Factor

叶片的吸光系数，该系数用于计算电子传递速率 ETR。吸光系数的默认值是 0.84，这适用于绝大多数高等植物。

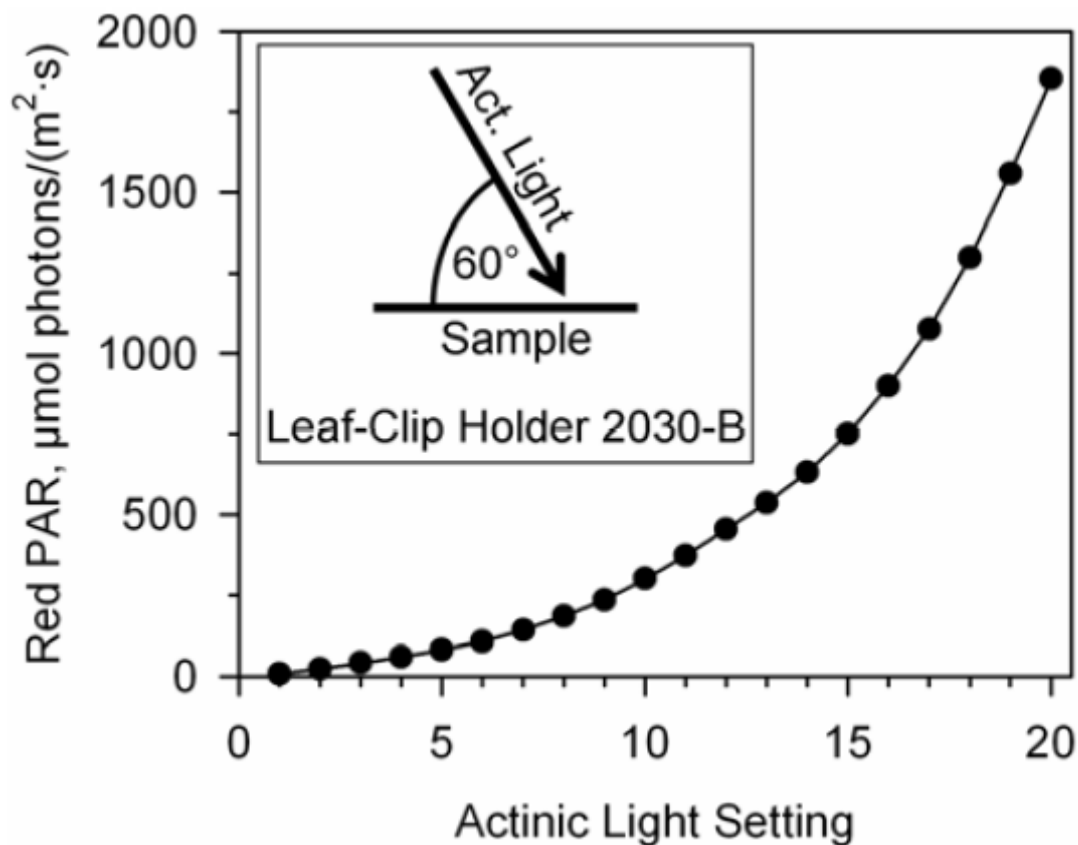


图 4.4 用 2030-B 时样品处红色光化光光强和光强梯度设置间的关系

AL Current/PAR Lists

PamWin-3 在安装时自动把<default_60.par>和表 4.1 中的其它几个光强列表文件复制到 <\PamWin-3\Data_2500>目录下。光强列表文件里包含了不同光几何学下内置红/蓝光源的输出信息。

第一次运行 PamWin-3 时程序自动加载<default_60.par>。<default_60.par>文件中对应的是用 2030-B，把光纤以 60° 角插到底时样品处接收到的光强。以后每次启动时程序会自动最近一次用过的光强列表文件。

如果<\PamWin-3\Data_2500>目录下没有<default_60.par>文件时，仪器将使用出厂时的设定值（factory values are used）。注意，在所有光强列表中当光化光光强设为 0 时表示关闭光化光，此时光化光的强度是测量光的强度（频率设为 10 Hz 或 100 KHz）。

未接外置光量子传感器时（或者在“General Settings”中通过取消<PAR sensor>来关闭光量子传感器），PamWin-3 计算的光强为从光强列表中读取的数值加上测量的光强。

表4.1 默认红蓝光源光强列表文件

File Name	Sample Holder	Optical Geometry*
default_60.par	Leaf-Clip Holder 2030-B	60°
	Distance Clip 2010-A	60°, 2 mm distance ring
	Arabidopsis Leaf Clip 2060-B	60°, 6 mm distance ring
default_90.par	Leaf-Clip Holder 2030-B	90°, 4 mm distance ring
default_90_2060.par	Arabidopsis Leaf Clip 2060-B	90°, 2 mm distance ring
default_DLC.par	Dark-Leaf-Clip DLC-8	90°
default_KS.par	KS-2500 Suspension	Cuvette 90° and 4 mm

图 4.4 显示了光强列表文件中光强和设置梯度间的关系图。每个光强列表文件里都包含了光学几何学信息，可以通过点击  图标查看。

每个光强列表都包含 3 列：<AL>光强设置梯度；<Current>表示当前 LED 的相对电流大小（最大值为 255）；<PAR>列出了光强值（ $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ）。红光光源的光强值和电流值线性相关，而蓝光光源的光强值和电流值半线性相关（quasi-linearly）。如果要更改电流值或者光强值，在相应区域点击并输入新的值即可。新的光强列表可以通过  图标注释， 图标保存， 图标打开。

Mode

<VIEW>命令将程序转换到离线模式，通过点击<MEASURE>回到测量模式。<VIEW>命令和<MEASURE>命令等效于<General Settings>窗口的 **VIEW-mode** 和离线时的 **MEASURE-mode** 按钮。

Service

<Service>用来进行仪器的固件升级（安装到仪器内部处理器上的软件被称为固件）。新版固件升级程序由 Walz 公司提供。可以通过点击<Read Firmware Version>查看当前固件版本。

注意：为防止固件升级时发生错误，在升级固件时请将电池充满电，如果出现错误请与 Walz 公司联系。

点击<Controller Service>进行固件升级的操作。PAM-2500 包括两个可编程的处理器 RISC 和 TINY。

a) RISC

点击 **Program RISC** → **Read HexFile** → **Download HexFile**。接下来点击 **Program RISC**（这一步需要等一段时间）。最后通过点击 **OK** 完成操作。

注意：在执行下一步前关掉并重启 PamWin-3 程序！！（Before proceeding, close and restart the PamWin-3 Program!）

b) TINY

点击 **Program RISC**，再点击 **Read HexFile** → **Download HexFile** → **Program RISC**。最后通过点击 **OK** 完成操作。关掉并重启 PamWin-3 程序。

<Trigger out with SP>该项功能在每个饱和脉冲时输出一个 5 V 的触发脉冲波。见 4.3.1.8 对 AUX 连接器触发针和触发脉冲的设置的描述。

Help

打开帮助文档。

4.3.1.2 测量光频率（Measuring Light Frequency）

在<General Settings>窗口设置测量光频率，各个图标对应的设置如表 4.2。

表 4.2 测量光频率

Icon		Range
MF-L	低频测量光	5 - 5000 Hz
MF-H	高频测量光	1000 – 100 000 Hz *

* 仪器的最大分辨率 200 000 Hz 在<Fast Acquisition Analysis Mode>

4.3.1.3 诱导曲线参数（Induction Curve Parameters）

PamWin-3 定义了三个和诱导曲线相关的时间参数（图 4.3）

Delay, seconds: 设定测量 Fv/Fm 后到打开光化光的间隔时间，默认为 40 S。

Clock, seconds: 设定在执行光化光期间相邻两个饱和脉冲之间间隔时间。

Width, seconds: 设定光化光持续时间。

4.3.1.4 全局设置（Global Settings）

Settings

Default 恢复默认设置（<Walz2500.DEF>）

Open User Settings 打开以前存储的设置文件

Save User Settings 将当前设置保存到设置文件（*.DEF）

Write To Report 将当前设置写入报告文件（见 4.4.3.1 中各命令的缩写）

Other Commands

Zoff 对荧光信号进行调零

Exit PamWin 关闭 PamWin-3 软件

4.3.1.5 光量子传感器控制（PAR Sensor Control）

选中<PAR Sensor Control>后打开外置光量子传感器，取消选择<PAR Sensor Control>后关闭外置光量子传感器，此时光强值从内置光强列表文件中读取。

4.3.1.6 F_o , F_o'

样品暗适应后点击 **F_o** 即把当前荧光值 F_t 记作 F_o 。

C/ F_o 代表的是恒定荧光产量（来自 PS I）对测量的 F_o 值的贡献量。只有激活了 F_o' 模式并且非光化学淬灭引起 F_o' 降到 F_o 值以下时才能评估 C/ F_o 的值。

F_o' 代表的是仪器测量的光下最小荧光（而不是计算的）。选中 F_o' 复选框便激活了 F_o' 模式，即在每个饱和脉冲后关闭光化光打开<PS I Light>光优先激发 PS I，使 PS II 反应中心迅速开放，光化学淬灭达到最大荧光产量达到最小。<PS I Light>光强可以在<General Settings>窗口进行设定（见<Field Screen>）。

$\sim F_o'$ 代表的是程序计算推导的光下最小荧光（而不是实际测量的）。 $\sim F_o'$ 是根据 Oxborough and Baker (1997) 的公式从 F_o , F_m 和 F_m' 推导而来（见 5.1.2）。

4.3.1.7 标签栏 (Tab Bar)

PamWin-3 软件中每个激活的窗口都会在<Tab Bar>区显示一个对应的标签，点击标签可以前端显示相应的窗口。

4.3.1.8 脉冲，短期光照 (Pulses, Short Term Illuminaion)

PamWin 高级应用可以给样品提供其它额外的光照，也可以通过定义一个触发信号来控制外部设备。

这些短时间事件的持续时间从几个微秒到 1 秒，他们分别是：

ST 单周转闪光，持续事件为 5~50 μ S 可调，光强为 10000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 。

MT 多周转闪光，持续事件为 1~300mS 可调，光强为 10000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 。

TR Pulse 向 AUX 插口传送一个 5 V 的触发脉冲，脉冲持续时间 10 μ S 到 1000mS 可调。

通过右键点击 **ST**，**MT**，或 **TR Pulse** 打开<Pulse Widths>设置每个短时间事件的持续时间。

在<Pulse Widths>窗口选中 **S+H off** 将取样保持电路放大器 (sample-and-hold amplifier) 暂时短期关闭，短期关闭取样保持电路放大器 (sample-and-hold amplifier) 可以防止由于执行单周转闪光而人为引起的信号的增大。关闭的时间长度从 **Extended S+H off time** 下拉列表中选择。

TR 手动控制向外接设备发送一个触发。

AL+Y 照射一段时间光化光（光强和持续时间在 **General Settings** 中设置）然后进行饱和脉冲分析。

FR+Y 执行的程序和**AL+Y** 相同，只是把光化光换成了<PS I light>。

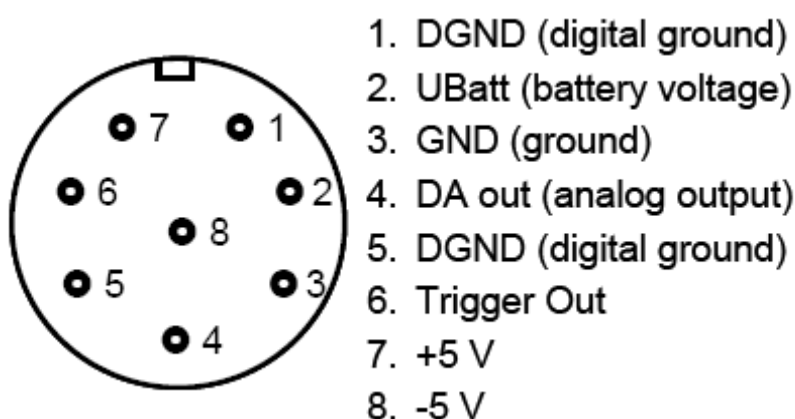


图 4.5 AUX 接口

4.3.1.9 模式 (Mode)

切换 PAM-2500 工作模式为 SP-Analysis 和 Fast Acquisition 或者离线模式 (View mode)。

4.3.1.10 定时事件 (Clock Event)

有 6 个测量程序可以设置成每隔一段时间重复执行。程序间隔时间为 3-900S 可调，但是要确保程序间隔时间比单个程序执行所花的时间长一些。这 6 个测量程序分别是：

SAT-Pulse: 饱和脉冲分析

AL: 照射光化光

AL+Y: 照射一段时间光化光并进行饱和脉冲分析

FR+Y: 照射一段时间<PS I Light>并进行饱和脉冲分析

Light Curve: 光响应曲线

Slow Induc.: 诱导曲线

4.3.1.11 新记录 (New Record)

新建一个报告文件。

4.4 饱和脉冲模式 (SP-Analysis Mode)

4.4.1 慢速动力学窗口 (Slow Kinetics Window)

<Slow Kinetics>窗口的主要功能是实时显示荧光值 F_t 和从饱和脉冲分析得到的参数 (曲线的形式显示) (图 4.6)。

可以通过 File 菜单里的<Save as pws-file>命令将荧光曲线数据保存为*.pws 格式文件, 在 View 模式下可以打开 pws 格式文件。

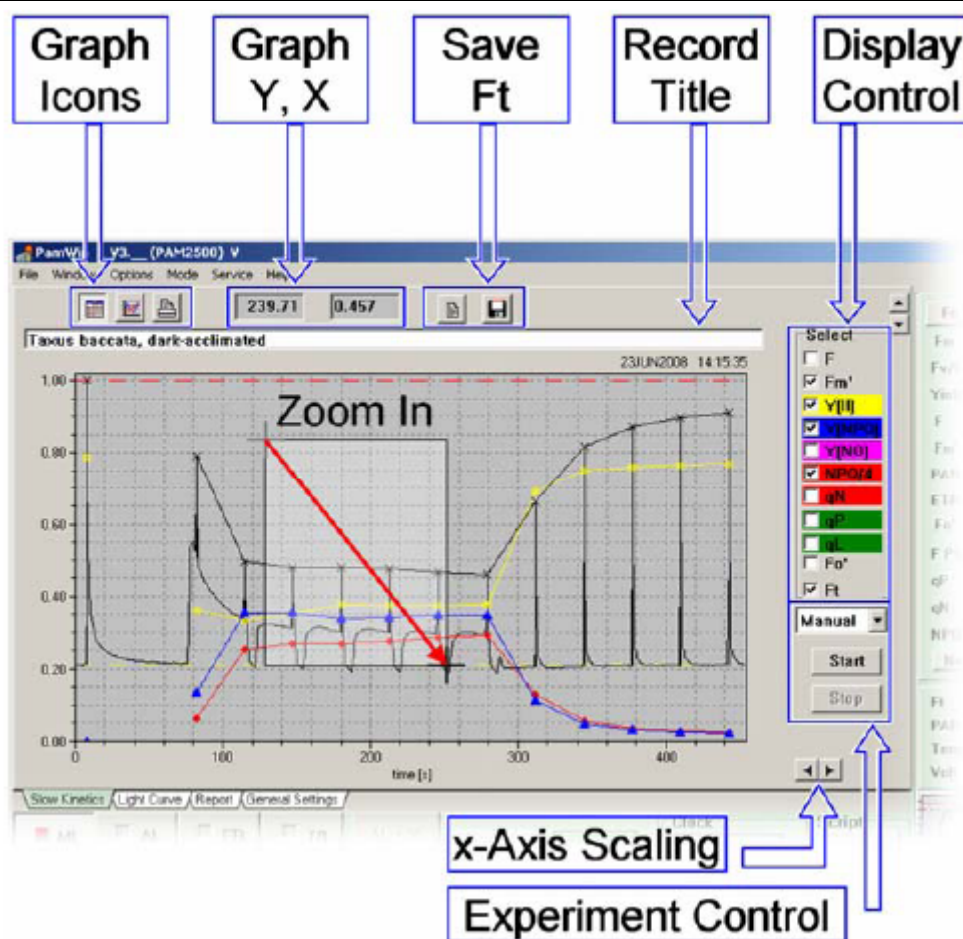


图 4.6 慢速动力学窗口

4.4.1.1 图标 (Graph Icons)

图 4.6 中从左到右三个图标的功能分别是：控制显示网格、自动调整两个坐标轴和打印图形。

4.4.1.2 图形 Y, X (Graph Y, X)

显示的是当前鼠标所处位置的在程序中的时间 (S, 左边), 和相对数值 (右边)。

4.4.1.3 保存 Ft (Save Ft)

点击  按钮保存当前 Ft 图像 (功能等同于<File>菜单的<Save as pws-file>)。点击  按钮打开已经保存的文件。

4.4.1.4 显示控制（Display Control）

实时显示荧光值 F_t 和从饱和脉冲分析得到的参数（曲线的形式显示）（图 4.6）。

4.4.1.5 X 坐标轴调整（X-Axis Scaling）

通过点击  调整横坐标的大小。

在图形区按住鼠标左键拖动鼠标可以放大选中的区域，在图形区的任何地方单击鼠标右键都可以自动调整坐标轴将所有图形都显示出来。

4.4.1.6 实验控制（Experiment Control）

从下拉菜单选择要执行的程序，点 / 开始或结束这个程序。数据被存储到报告文件。

4.4.2 光响应曲线（Light Curve）

光响应曲线的详细信息见 5.5。

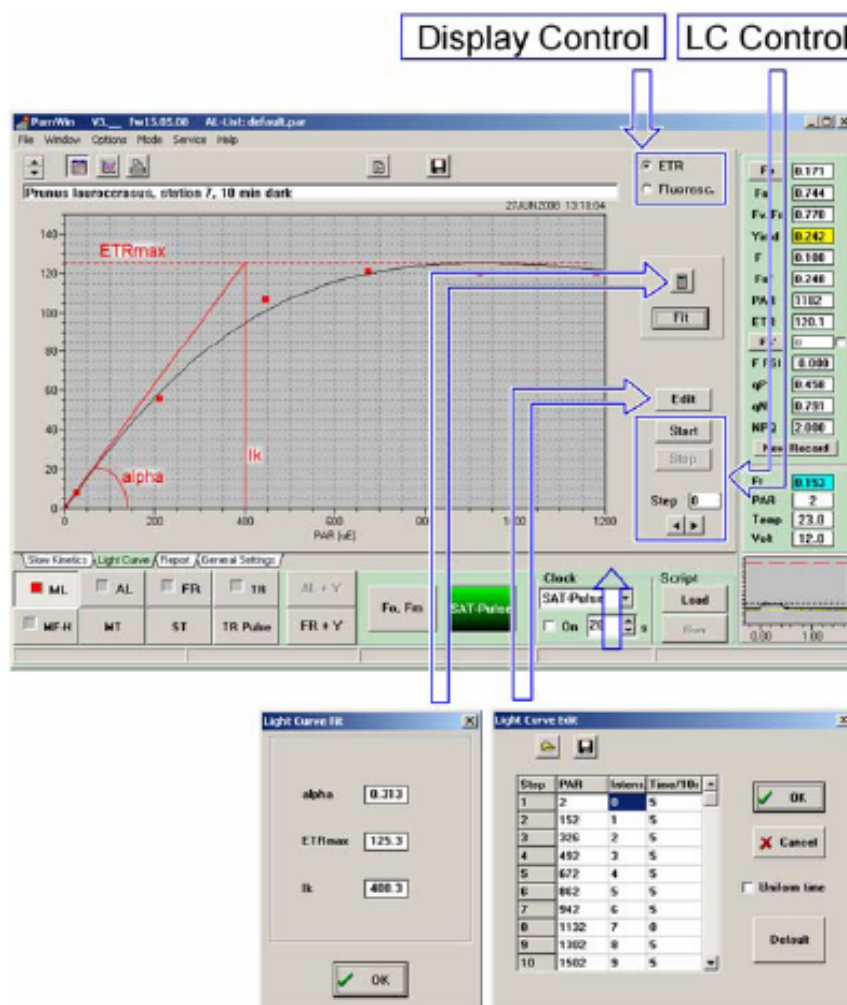


图 4.7 光响应曲线窗口

4.4.2.1 显示控制（Display Control）

选中 **Fluoresc.**后在图形区显示荧光比值参数，选中 **ETR** 后将显示 **ETR** 对 PAR 的曲线图。和传统的光响应曲线不同，ETR 对 PAR 曲线测定速度很快，在每个光强下 ETR 并没有达到稳态，因此这个光曲线也叫快速光曲线。

4.4.2.2 光响应曲线控制（Light Curve Control）

点击 **Start** / **Stop** 开始或结束一个光响应曲线程序。

4.4.2.3 光响应曲线编辑（Light Curve Edit）

编辑光响应曲线的程序，可以将编辑好的程序保存为*.lcp 格式的文件，也可以导入以前编辑好的光响应曲线的程序。

可以设置光强、每个光强光化光的持续时间，当持续时间设为“0”时，程序执行到这一步便会停止，例如，将第 9 步的持续时间设为“0”，那么程序执行时只会执行 1-8 步设置的光强，然后停止。

注意：此时显示的实际光强值是从内置光强列表文件中读取的。如果实验室使用了 2030-B，且激活了外置光量子传感器时，仪器记录的是实际测量值。

4.4.2.4 光响应曲线拟合（Light Curve Fit）

采用 Eilers and Peeters (Ecological Modelling 42(1988) 199-215)的公式进行光响应曲线的拟合，3 个拟合参数如下：

α ，单位：electrons/photons，快速光曲线的初始斜率，反映了光能利用效率。

ETR_m，单位： $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ，最大电子传递速率。

E_k，单位： $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ，最小饱和光强（半饱和光强），反映了样品对强光的耐受能力。

4.4.3 报告文件（Report）

4.4.3.1 报告文件数据排列（Report Data Arrangement）

<General Settings>窗口的<Write to Report>命令执行时在报告文件中写入一行包含 16 个参数的数据（表 4.3）。

4.4.3.2 报告文件头和最后一行（Record Header and Last Line）

第一行：报告标题

第一行为报告文件的标题。可以在<Slow Kinetics>或<Light Curve>窗口上部输入实验注释，输入的实验注释也被记录到报告文件的第一行。

第二行和第三行：F_o, F_m

第二行和第三行显示的是 F_o , F_m 和 F_v/F_m , 如果用<Induction Curve>或<Induction Curve Recovery>程序进行测量时, 在第二行会加入<IC>或<IC+>缩写。

注意: 如果实验时第一个饱和脉冲不是测定 F_o 和 F_m , 第二行和第三行就不显示 F_o , F_m 和 F_v/F_m , 如果在实验的过程中测定了 F_o 和 F_m , 那么后来测定的 F_o 和 F_m 也不会再记录到第二行和第三行, 但是仍然使用后面测定的 F_o 和 F_m 来计算其他参数。

最后一行: 荧光曲线的状态

这一行描述了当前实验过程中有没有测量原始荧光曲线。

4.4.3.3 纪录列 (Record Columns)

在测量模式下记录图表 (Record chart) 包含 12 列 (在<View>模式下可以看到更多的数据)。下面对图 4.8 中标注的数据列分别进行注释 (测量模式下)。

- 1、日期和时间
- 2、饱和脉冲光的编号, 如果第一饱和脉冲测量的是< F_o , F_m >, 将不显示编号 1
- 3、测量光的强度
- 4、温度 $^{\circ}\text{C}$
- 5、光化光强度
- 6、 F_o (第一行) 和 F (接下来的数据) (电压值)
- 7、光合电子传递速率
- 8、PS II 的最大量子产量 (F_v/F_m)、实际量子产量 ($Y(\text{II})$)
- 9、光化学淬灭系数, 这个值为 1 时说明没有正确测量 F_o , F_m
- 10、非光化学淬灭系数, 这个值为 0 时说明没有正确测量 F_o , F_m
- 11、 F_m (第一行) 和 F_m' (接下来的数据) (电压值)
- 12、 F_o' , 如果没有激活 F_o' -mode, 这一列为空
- 13、电池电压

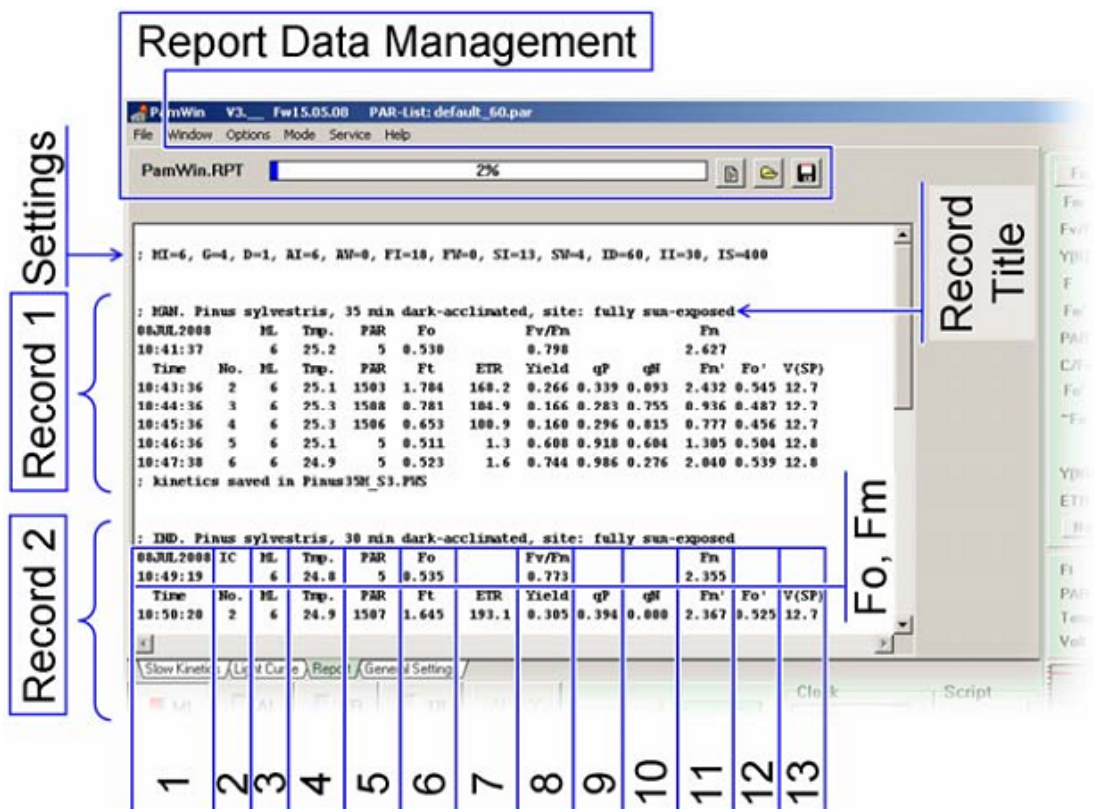


图 4.8 报告文件窗口

表 4.3 仪器设置报告

Abbreviation	Parameter
AI	Actinic Light Intensity
AW	Actinic Light Width
BI	Blue Light Intensity
BW	Blue Light Width
D	Damping
FI	Far Red Light Intensity
FW	Far Red Light Width
G	Gain
H	High Measuring Frequency
ID	Slow Induction Delay
II	Slow Induction Clock
IS	Slow Induction Actinic Light Width
L	Low Measuring Frequency
MI	Measuring Light Intensity
SI	Saturating Pulse Intensity
SW	Saturating Pulse Width

4.4.3.4 报告文件数据管理

在报告文件窗口，<File>菜单包括 4 个子菜单，分别是：

Open Report

打开以前存储的报告文件。

Save Report

将当前数据保存为*.rpt 格式的文件。

Clear Report

删除当前报告文件中的所有数据，并开始新的记录。

Print Report

打印当前报告文件。

4.5 快速获取模式（Fast Acquisition Mode）

PamWin-3 的快速获取模式最大时间分辨率为 10 μ S，用来记录快速荧光上升动力学。

4.5.1 快速设置 (Fast Setting)

4.5.1.1 基础设置 (Basic Setting)

MF-max

设置测量光的最大频率（图 4.9），测量光越高仪器的信噪比越低，但是测量光强也随着频率的升高而升高，从而会影响到光合作用。

Rate/Points

<Fast Kinetics>的总时间由采样速率和采样点的数目共同决定。在<Fast Kinetics>模式下执行一次测量最多可以采集 128000 个时间点的数据。

Target Averages

设置是否对<Fast Kinetics>模式中采集的数据点取平均值（相邻的数据点），并设置每几个点取一次平均值。

4.5.1.2 菜单栏 (Menu Bar)

<Fast Kinetics>模式下的菜单和<SP-Analysis>模式下（见 4.3.1.1）的菜单基本相同。

4.5.1.3 触发设置 I 和 II (Trigger Settings (I) and (II))

一共有 6 个工具标签来对触发信号进行设置（图 4.9，图 4.10）。

一共有 8 个不同的事件可以被触发（见 Trigger Settings(II)和表 4.4）。在这些事件中，单周转闪光（ST）的时间应该非常短（只让 PS II 反应中心完全周转一次），因此在设置 ST 时只有<High Pulse>和<All Low>按钮可用。

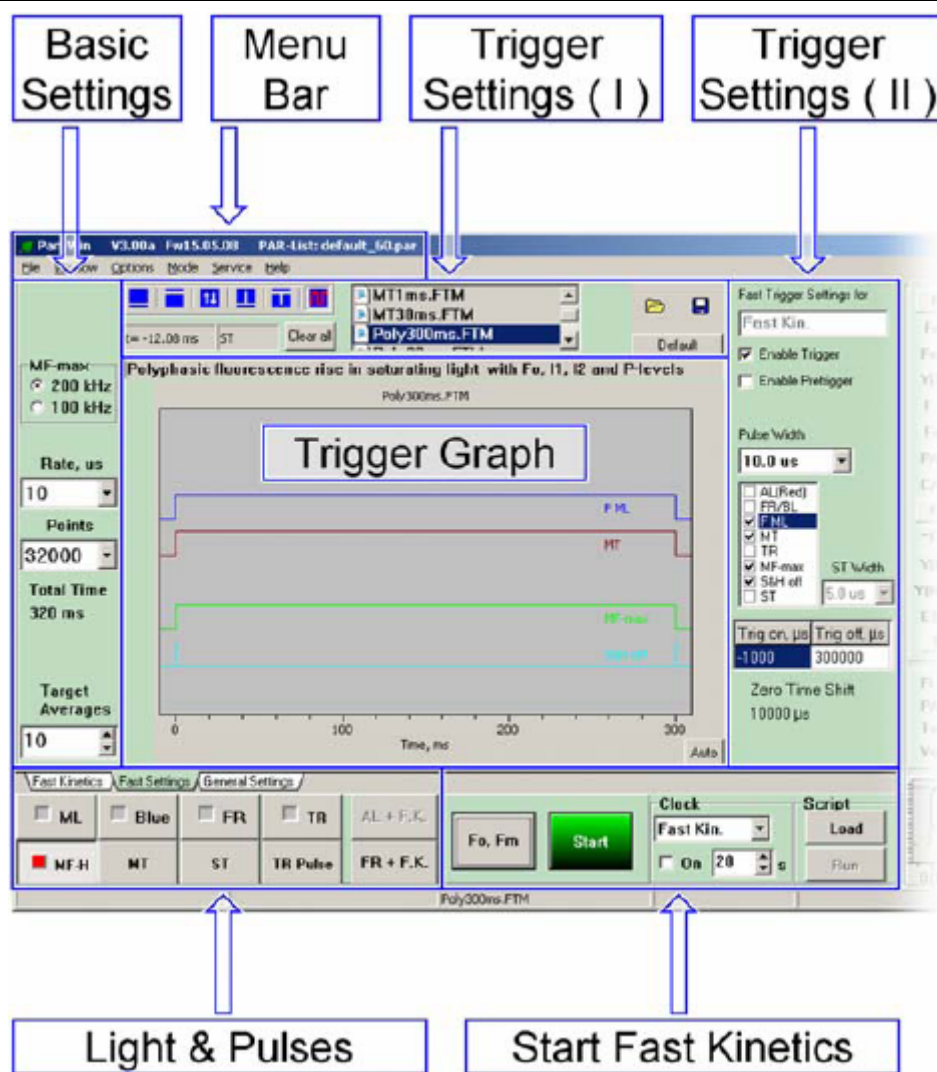


图 4.9 快速设置窗口

表 4.4 触发图：复选框面板

Checkbox Label	Action	Comments
AL(Red)	Red actinic light	Intensity - as defined for <Act. Light> in <General Settings>
FR/BL	Far red light/Blue light	FR/Blue selection: <General Settings>. Intensity as defined for <PS I Light> in <General Settings>
F ML	Measuring light	Frequencies (MF-L. and MF-H.) and intensity: <General Settings>. Measuring light settings at kinetics start are controlled by ML and MF-H buttons.
MT	Multiple turn-over flash	Intensity - as defined for <Sat. Pulse/MT> in <General Settings>
TR	Trigger out	See Section 4.3.1.7
MF-max	Maximum frequency of measuring light	Frequency as defined in <Fast Settings> window (MF-max)
S&H off	Sample and hold circuit off	Suppresses artifacts caused by extreme intensity variations of non-measuring light
ST	Single turn-over flash	Always at maximum intensity (about 125 000 $\mu\text{E}/(\text{m}^2\cdot\text{s})$)

其他所有事件都可以被脉冲和信号转移（signal shifts）触发。要设置一个脉冲：

- 通过点击标签上的复选框选择一个事件（图 4.9 Trigger Settings(II)）
- 点击相应的框（box）激活事件
- 点击<High pulse>标签
- 从下拉菜单选择时间间隔
- 点击<Trig on, us>下面的区域，输入脉冲开始采集数据点的时间

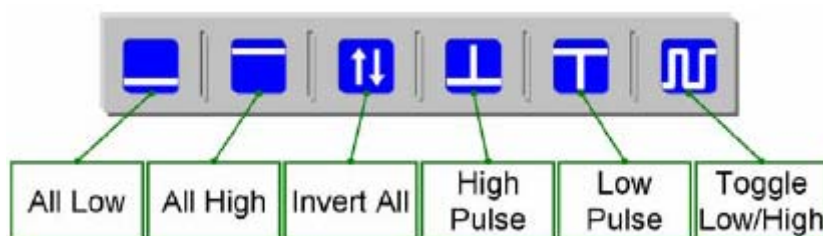


图 4.10 触发工具标签

4.5.1.4 光源和脉冲（Light & Pulses）

AL + F.K. 打开光化光随后执行当前脉冲

FR + F.K. 打开<PS I Light>光随后执行当前脉冲

4.5.1.5 开始快速动力学（Start Fast Kinetics）

Fo, Fm 测量 Fo, Fm

Start 开始当前触发

4.5.2 快速动力学（Start Fast Kinetics）

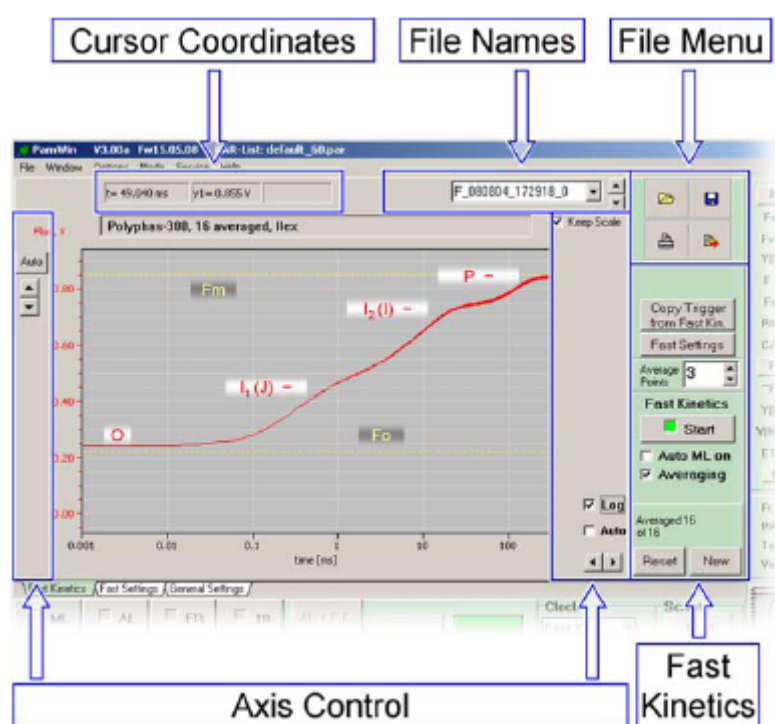


图 4.10 快速动力学窗口

4.5.2.1 光标坐标（Cursor Coordinates）

显示鼠标光标的位置。

4.5.2.2 文件名（File Names）

当前快速动力学曲线的文件名，格式为 F_日期_时间_0。

4.5.2.3 文件菜单（File Menu）

将实验数据保存成*.pflk 格式或者导出成 ASCII 格式的文件。

4.5.2.4 快速动力学（Fast Kinetics）

Copy Trigger from Fast Kin. 用于重复当前显示的动力学曲线

Fast Settings 打开快速设置窗口

Average Point 对<Fast Kinetics>模式中采集的数据点取平均值（相邻的数据点）时设置每几个点取一次平均值

Start 开始当前触发测量

AUTO ML ON 选中后在开始<Fast Kinetics>时自动打开测量光

4.6 查看模式（View Mode）

查看模式可以在未连接 PAM-2500 荧光仪时使用，当然连接荧光仪时照样可以使用。主要用于数据的查看和分析。

4.6.1 图标栏（Icon Bar）

图标的功能包括注释数据文件、打开数据文件、保存数据、将数据导出成 ASCII 码格式文件、将数据导出到系统粘贴板等。

4.6.2 侧栏（Sidebar）

详细功能见图 4.11 中的列表。

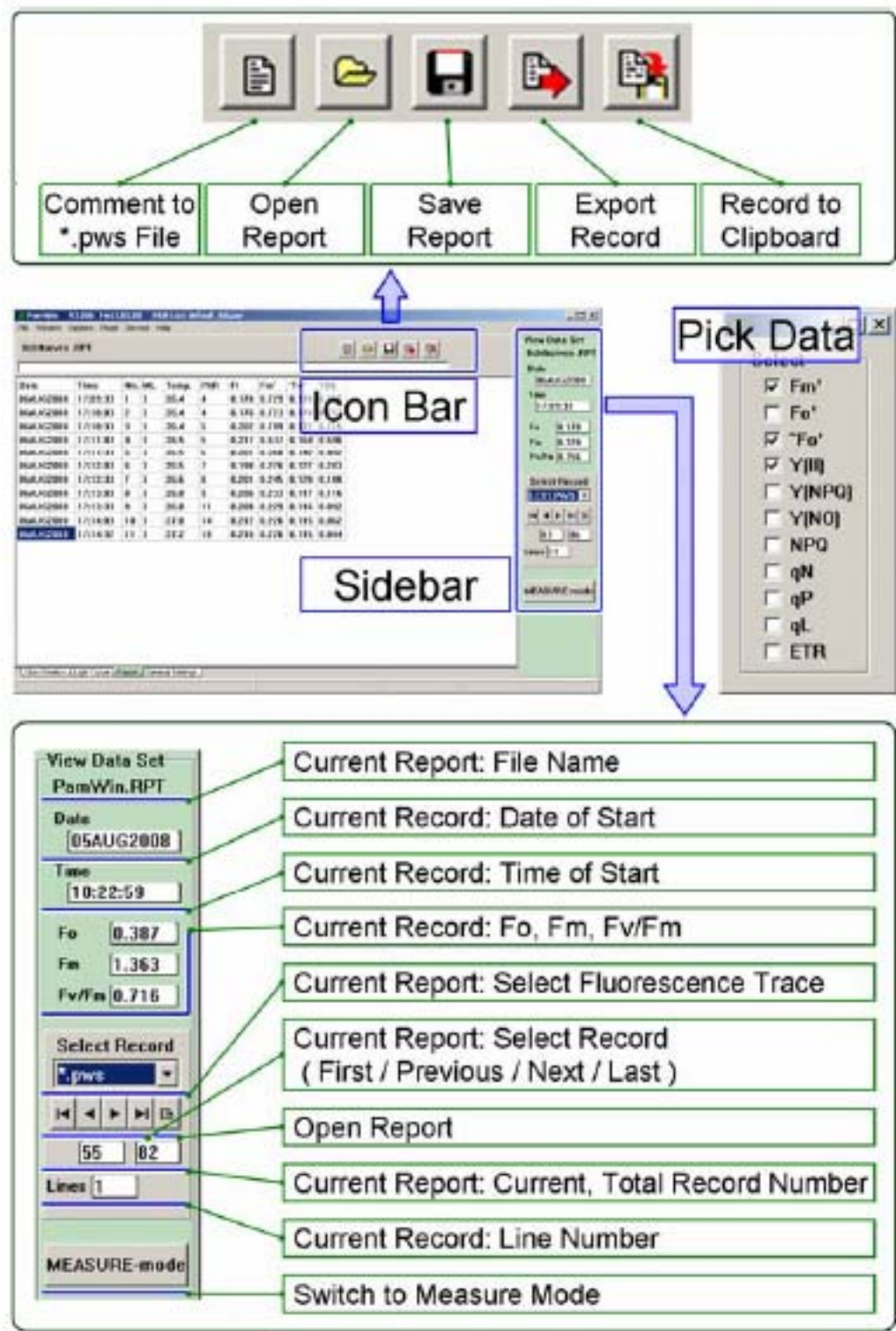


图 4.11 查看模式窗口

4.6.3 挑选数据（Pick Data）

选中在报告文件窗口显示的数据。

4.7 脚本文件（Script Files）

脚本文件用来编制一些测量程序来完成一些复杂的测量或者是自动测量。使用脚本程序大大扩展了 PAM-2500 的功能，你可以编写自己需要的测量程序。

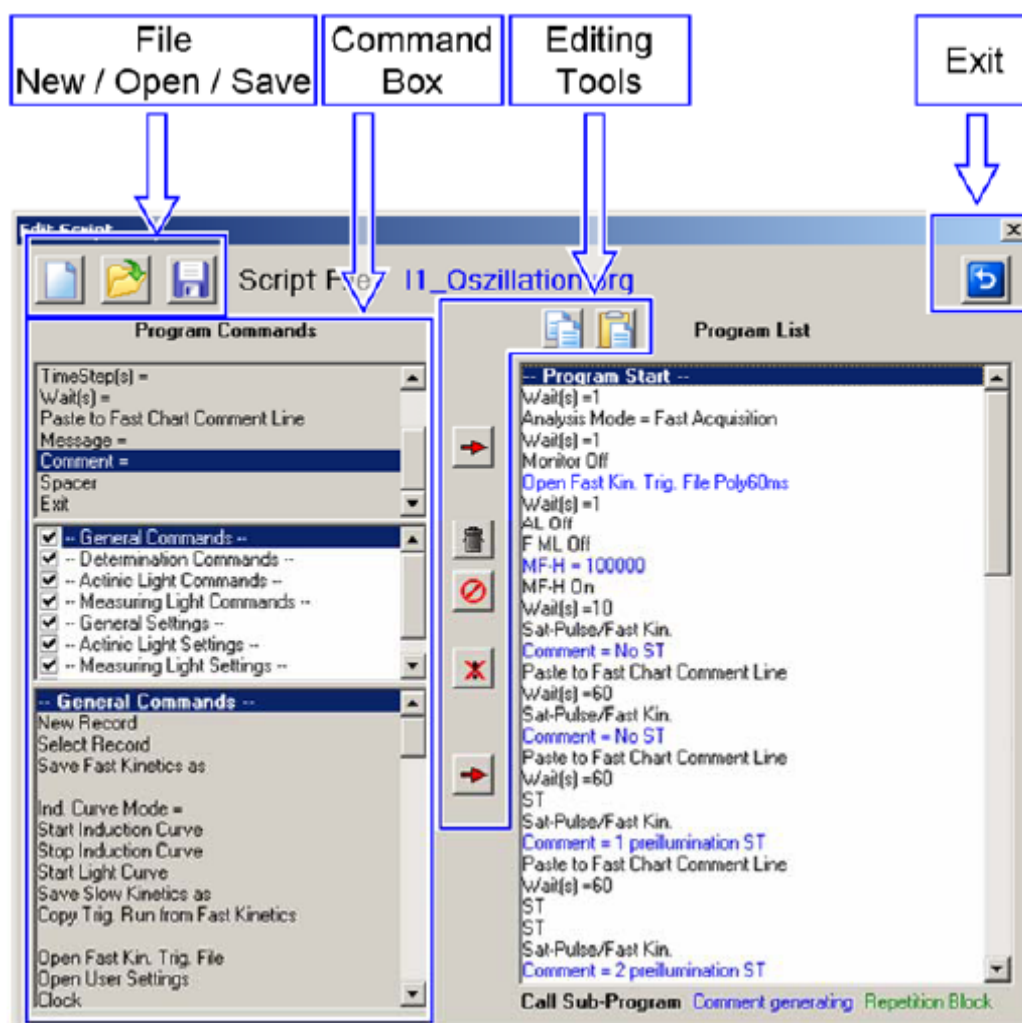


图 4.12 脚本程序窗口

按照下面的步骤创建一个新的脚本程序：

- 点击 **Load** 图标打开一个读取脚本文件的对话框
- 点击 **OPEN** 但不要选择任何文件，而是点击 **Cancel**，然后输入要创建的脚本程序名。
- 也可以打开一个现有的脚本文件，然后点击 <New Script File> 新建一个脚本文件。

4.8 编写脚本程序

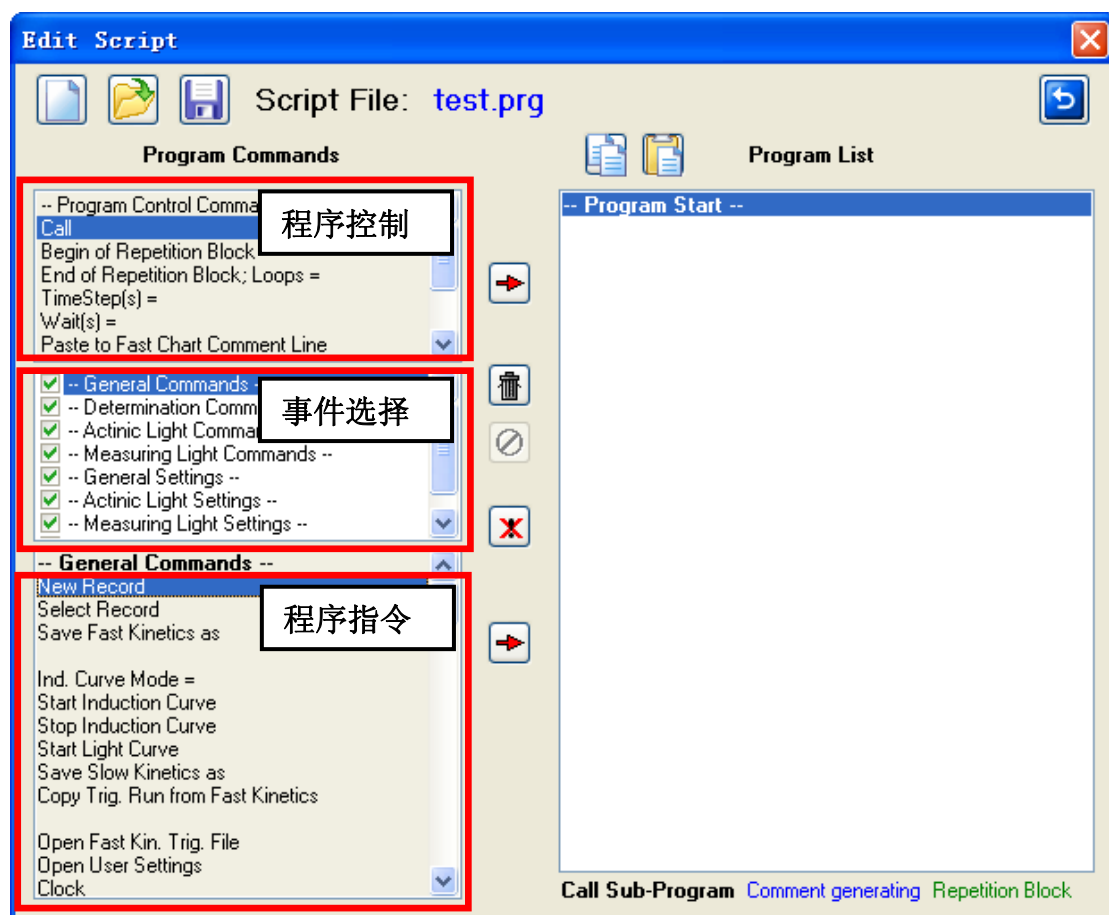


图 4.13 脚本程序窗口

为了描述方便同时根据各自功能现将编程窗口的左半部分分为 3 个区，分别是：程序控制区、事件选择区和程序指令区，下面对各个区的功能进行简要介绍（图 4.13）。

程序控制区

该区域的命令主要是控制整个程序的运行情况，现将其中包含的主要命令进行简要介绍。

Call 从本机硬盘上调取一个已经编辑好的脚本程序文件

Begin of Repetition Block 和 “End of Repetition Block; Loops=” 连用，开始一个循环程序，循环执行设定 Begin of Repetition Block 和 End of Repetition Block; Loops= 之间的命令，Loops=是指该循环的次数。

TimeStep(S)= 设置当前步骤的时间

Wait(S)= 设置等待时间，即在等待一段时间后再执行下一个命令

Comment= 对脚本文件的注释

Spacer 空格

Exit 终止当前程序

事件选择区

点击响应事件后会在“程序指令区”显示和该事件对应的指令。

程序指令区

显示和“事件选择区”对应的程序指令，通过这些指令可以控制 PAM-2500 执行各项操作。

PAM-2500 的快速光曲线（RLC）程序模板

程序	解释
-- Program Star --	脚本程序开始
F ML ON	打开测量光
AL ON	打开光化光
AL-Int. =1	设置光化光强度为 1
Wait(s) =10	等待 10 S
Sat-Pulse/Fast Kin.	执行饱和脉冲
AL-Int. =2	将光化光强度升高到 2
Wait(s) =10	等待 10 S
Sat-Pulse/Fast Kin.	执行饱和脉冲
.....	逐渐升高光化光强度
AL-Int. =9	将光化光强度升高到 9
Wait(s) =10	等待 10 S
Sat-Pulse/Fast Kin.	执行饱和脉冲
AL Off	关闭光化光
F ML Off	关闭测量光
Exit	退出程序

我们提供了一个动画以快速光曲线的程序为例简单演示了脚本程序的具体编制方法，这里只是提供一个简单的例子，具体操作根据程序的不同而不同。动画下载地址：<http://www.zealquest.com/down.as>

5 参数符号及计算公式

5.1 相对荧光产量

5.1.1 样品暗适应后进行测定

F₀ 当 PSII 反应中心都处于开放状态时的最小荧光。

F_m 暗适应后执行饱和脉冲当 PSII 反应中心都处于关闭状态时的最大荧光产量。

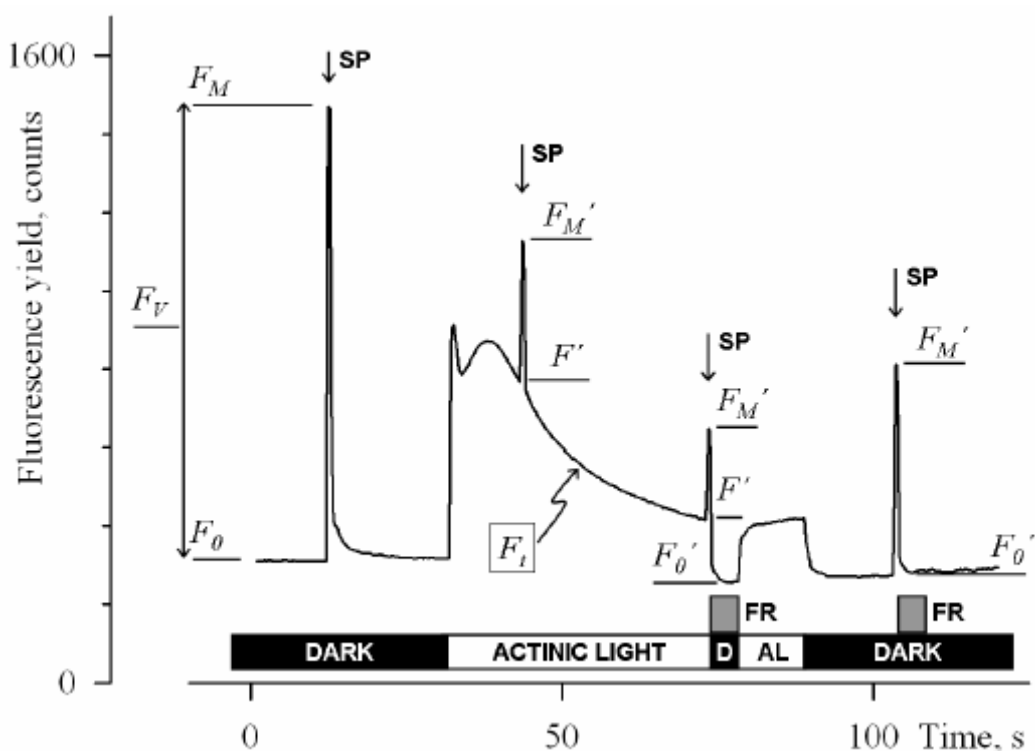


图 5.1 饱和脉冲分析。AL，光化光；D，黑暗；SP，饱和闪光；FR，远红光。

5.1.2 对光下样品进行测量

F₀' 光下最小荧光。当<Fo'-Mode>激活时，在饱和脉冲后仪器关闭光化光打开远红光优先激发 PSI，PSI 迅速从两个光系统间的电子递体得到电子，使 PSII 反应中心打开，此时荧光产量降到最低记为 F₀'（图 6.1 75S 时）。当<Fo'-Mode>未激活时，参考 Oxborough 和 Baker（1997）的方法计算 F₀'。计算公式如下：

$$Fo' = 1/(1/Fo-1/Fm+1/Fm')$$

Fm' 光下执行饱和脉冲当 PSII 反应中心都处于关闭状态时的最大荧光产量。

F' 执行饱和脉冲前的实时荧光产量。

5.2 荧光淬灭参数

叶片吸收的光能用于光化学反应和非光化学反应的两部分可以通过荧光产量的比值参数来进行区分。表 6.1 列出了 WinControl-3 中用到的所有用比值表示的荧光参数及计算公式。下面就各个参数进行简单的介绍。

表 6.1 用比值表示的荧光参数

定义及文献来源	计算公式
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$Fv/Fm=(Fm-Fo)/Fm$
Effective photochemical quantum yield of PS II (Genty et al., 1989)	$Y(II)=(Fm'-F')/Fm'$
Coefficient of photochemical fluorescence quenching (Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990)	$qP=(Fm'-F')/(Fm'-Fo')$
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer et al. 2004)	$qL=qP*Fo'/F'$
Coefficient of photochemical fluorescence quenching (Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990)	$qN=1-(Fm'-fo')/(Fm-Fo)$
Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman, 1990)	$NPQ=Fm/Fm'-1$
Quantum yield of non-light induced non-photochemical fluorescence quenching (Kramer et al. 2004)	$Y(NO)=1/(NPQ+1+qL*(Fm/Fo-1))$
Quantum yield of light-induced (Δ pH and zeaxanthin-dependent) non-photochemical fluorescence quenching (Kramer et al. 2004)	$Y(NPQ)=1-Y(II)-Y(NO)$

Fv/Fm and Y(II) PSII 的最大量子产量（Fv/Fm）和实际量子产量（Y(II)）

这两个参数表示的都是 PSII 将吸收的光能转化成化学能的效率。测 Fv/Fm 前，样品必需经过充分的暗适应以确保 PSII 所有的反应中心都处于开放状态并且非光化学淬灭达到最小。不同植物的暗适应时间不同，阴生叶片和阳生叶片的暗适应时间也不相同。

Y(II)反映的是光下叶片的实际光能转化效率。只有当照光强度（光化光）达到一定水平时 Y(II)的信息才能真实的反映光合的状态，因为在光强很弱时卡尔文碳同化过程可能无法正常运转而 Y(II)可能会比较高。

qP and qL 光化学淬灭系数

这两个参数表示的是 PSII 中处于开放状态的反应中心所占的比例。其中 qP 是基于沼泽模型的（puddle model, Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990）。qL 是基于湖泊模型的（lake model, Kramer et al. 2004）。

qN and NPQ 非光化学淬灭参数。

这两个参数都和基于跨膜质子梯度和玉米黄质的非光化学淬灭相关。

Y(NO) and Y(NPQ) 非光化学淬灭的量子产量

这两个是 Kramer 等在 2004 年提出的新参数。Y(NPQ)是指 PS II 处调节性能量耗散的量子产量。若 Y(NPQ)较高，一方面表明植物接受的光强过剩，另一方面则说明植物仍可以通过调节（如将过剩光能耗散为热）来保护自身。Y（NPQ）是光保护的重要指标。Y(NO)是指 PS II 处非调节性能量耗散的量子产量。若 Y(NO)较高，则表明光化学能量转换和保护性的调节机制（如热耗散）不足以将植物吸收的光能完全消耗掉。也就是说，入射光强超过了植物能接受的程度。这时，植物可能已经受到损伤，或者（尽管还未受到损伤）继续照光的话植物将要受到损伤。Y（NO）是光损伤的重要指标。

$$Y(II)+Y(NO)+Y(NPQ)=1$$

5.4 相对电子传递速率

相对电子传递速率的计算公式如下:

$$ETR = PAR \cdot ETR\text{-Factor} \cdot P_{PS2}/P_{PPS} \cdot Y(II)$$

ETR 计算的基本思想是乘积, PSII 的实际量子产量, $Y(II)$ 乘以 PSII 吸收的光能。下面对公式中的各个参数进行简单介绍。

PAR 光合有效辐射

ETR-Factor 吸光系数

ETR-Factor 是指光合色素吸收光量子的比例。在可见光范围内 (400-700 nm) 高等植物吸光系数的经验值约为 0.84。不同样品的吸光系数可能不同, 当不同样品间相互比较时要考虑到这些差别。

P_{PS2}/P_{PPS} PSII 光合色素吸收的光量子占总光合色素吸收的光量子的比例

假设 PSII 和 PSI 接收的光量子数量是相同的, 即 $P_{PS2}/P_{PPS} = 0.5$ 。WinControl-3 用 0.5 做 P_{PS2}/P_{PPS} 的默认值。

5.5 光响应曲线

测量光响应曲线时<Light Curve>程序连续照射几个强度逐渐升高的光化光, 一般情况下每个梯度的光化光持续时间太短而不足以使光合作用达到稳态。因此, 此时的光响应曲线被称为快速光曲线(RLC)。和传统的光响应曲线不同, 快速光曲线反映了当前状态下光合作用的信息。通过对快速光曲线进行拟合, 可以得到以下几个主要参数:

- α , 单位: electrons/photons, 快速光曲线的初始斜率, 反映了光能利用效率。
- ETRm, 单位: $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 最大电子传递速率。
- E_k , 单位: $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 最小饱和光强 (半饱和光强), 反映了样品对强光的耐受能力。

PamWin-3 采用 Eilers and Peeters (1988) 的公式对快速光曲线进行拟合, 拟合公式如下:

$$ETR = \frac{PAR}{a \cdot PAR^2 + b \cdot PAR + c}$$

各个参数的计算公式分别是:

$$\alpha = \frac{1}{c}$$

$$ETR_{\max} = \frac{1}{b + 2 \cdot \sqrt{a \cdot c}}$$

$$I_K = \frac{c}{b + 2 \cdot \sqrt{a \cdot c}}$$

图 5.2 给出了几条快速光曲线的例子及拟合后的参数。

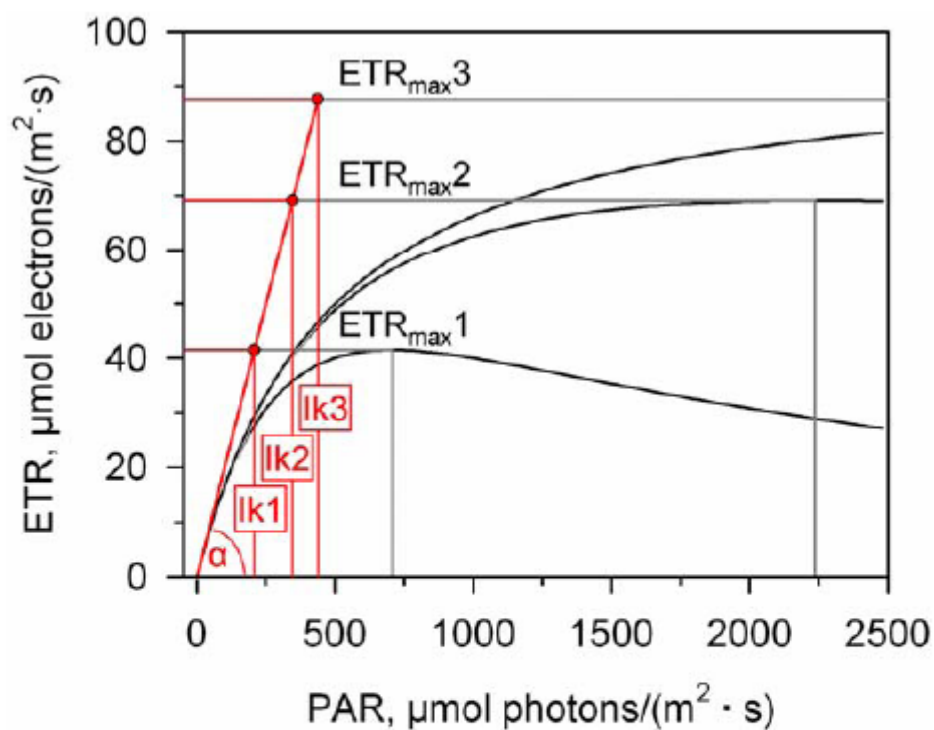


Figure 5.2: Three Exemplary Rapid Light Curves.

Curve	Parameter					
	a	b	c	α	$ETR_{\max}$	I_K
1	$1 \cdot 10^{-5}$	$1 \cdot 10^{-2}$	5	0.2	41	207
2	$1 \cdot 10^{-6}$	$1 \cdot 10^{-2}$	5	0.2	69	345
3	$1 \cdot 10^{-7}$	$1 \cdot 10^{-2}$	5	0.2	88	438

5.5.1 关于快速光曲线的文献

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