Application Note: 2201
August 2022

Using Handy PEA+ to measure Rapid Light Curves

A Novel Application

Hansatech Instruments

Using Handy PEA+ to measure Rapid Light Curves - a novel application

Summary

Rapid Light Curves (RLCs) have been used in photosynthesis research since the late 1990s, predominantly using Pulse Amplitude Modulation (PAM) fluorometers. This application note describes how to measure RLCs using the non-modulated Hansatech Instruments Handy PEA+. A separate note covers RLCs using M-PEA.

Light curves in photosynthesis research

Various types of light curves have been used for many years in the study of photosynthetic performance because they can assess both the plant's present photosynthetic capacity and potential activity across a range of ambient light intensities (Ralph & Gademann 2005).

P – E curves (or photosynthesis – irradiance curves, Figure 1) were traditionally made by the measurement of oxygen evolution rates at different ambient light intensities or from C isotope incorporation (Houliez et al 2017).

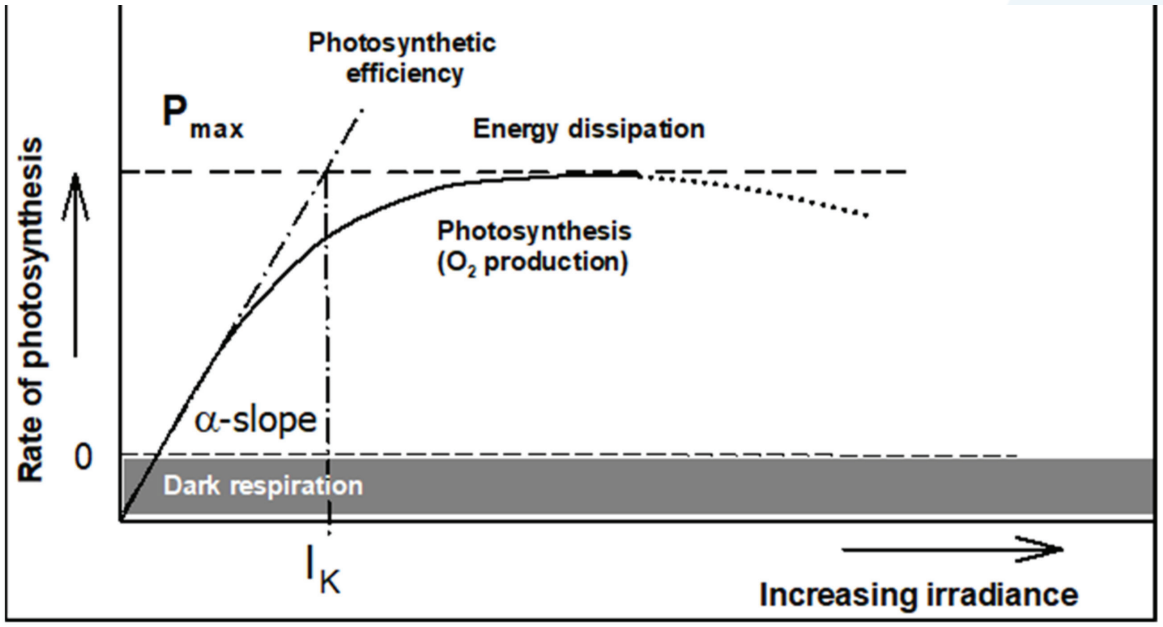

Figure 1. An illustration of a photosynthetic light-response curve, i.e., the dependency of the rate of photosynthesis on irradiance. The α-slope shows the maximum efficiency of photosynthesis (P - E). The intersect between the α-slope and maximum rate of photosynthesis P_{max} indicates the light saturation (I_K or E_K); surplus light energy over saturation is dissipated. At supra-optimum irradiance, the photosynthetic rate declines (dotted part of the light-response curve); this phenomenon is usually called down-regulation or photoinhibition (taken from Masojídek et al. 2021)

Steady-state light curves (SSLCs) are a chlorophyll fluorescence-based measurement protocol plotting the Electron Transport Rate (ETR) as a function of Photon Flux Density (PPFD) and are comparable to traditional *P – E* curves (Houliez et al 2017).

SSLCs take measurements of photosynthetic rates during several light steps at increasing intensities. The duration of each step is sufficiently long to achieve steady-state photosynthetic rates during that step. SSLC protocols can typically take anything from several minutes to a few hours to complete. Under field conditions, this presents significant challenges when comparing results between different plants, as varying factors such as time of day, associated diurnal changes of the plant and dynamic weather conditions must be considered (Rascher et al 2000).

Rapid Light Curves (RLCs) can be used to provide detailed information relating to the saturation characteristics of electron transport through photosystem II (PSII) in addition to overall photosynthetic performance (Ralph & Gademann 2005).

RLCs consist of a series of relatively short (<30s, typically 10s) light steps, with the light intensity increasing at each step. Each light step is interspersed by a saturating pulse where the ETR value is calculated. Unlike the *P* – *E* light response curves or the SSLC, RLC protocols do not achieve steady-state photosynthetic rates during the light steps. In contrast to *P* – *E* curves, which provide an indication of optimal state of photosynthetic performance independent of light history, RLCs indicate the current state of photosynthetic performance. Because steady-state conditions are not reached in RLCs, they reflect the light-acclimation state in the period leading up to the measurement and also longer-term light history (Ralph & Gademann 2005).

With ETR plotted against PPFD, RLC show three distinct regions (Figure 2):

1. **Light-limited region**
   Photosynthetic rates are limited by low light levels in the light-limited region. The parameter α indicates the slope of the rise of ETR vs. PPFD and is proportional to efficiency of light capture (effective quantum yield or ΦPSII) (Schreiber, 2004).
2. **Light-saturated region**
   During this phase, the capacity of the electron transport chain limits the electron transport rate. The ETR vs. PPFD curve reaches a plateau where maximum ETR occurs (denoted by the parameter ETR_{max}) (Schreiber, 2004). The minimum saturating irradiance, denoted by the parameter E_k (sometimes referred to as I_k), is determined by finding the intercept of α and ETR_{max} (Sakshaug et al., 1997) and can be related to quenching. Below E_k, photochemical quenching is the dominant pathway whereas non-photochemical quenching is dominant above E_k (Henley, 1993).
3. **Photoinhibited/Down-regulated region**
   In this region, where the plant is subjected to supra-saturating light intensities, the ETR vs. PPFD curve often tends to decline, which could be associated with photoinhibition (Henley, 1993). This effect would be more likely to occur with traditional *P* – *E* or SSLCs, where steady-state photosynthetic rates are achieved. However, as steady-state is not achieved in RLC protocols, there isn't normally sufficient time for photodamage to occur. It has been suggested that the decline of ETR at supra-saturating light intensities could be linked to dynamic down-regulation of PSII (White and Critchley, 1999).

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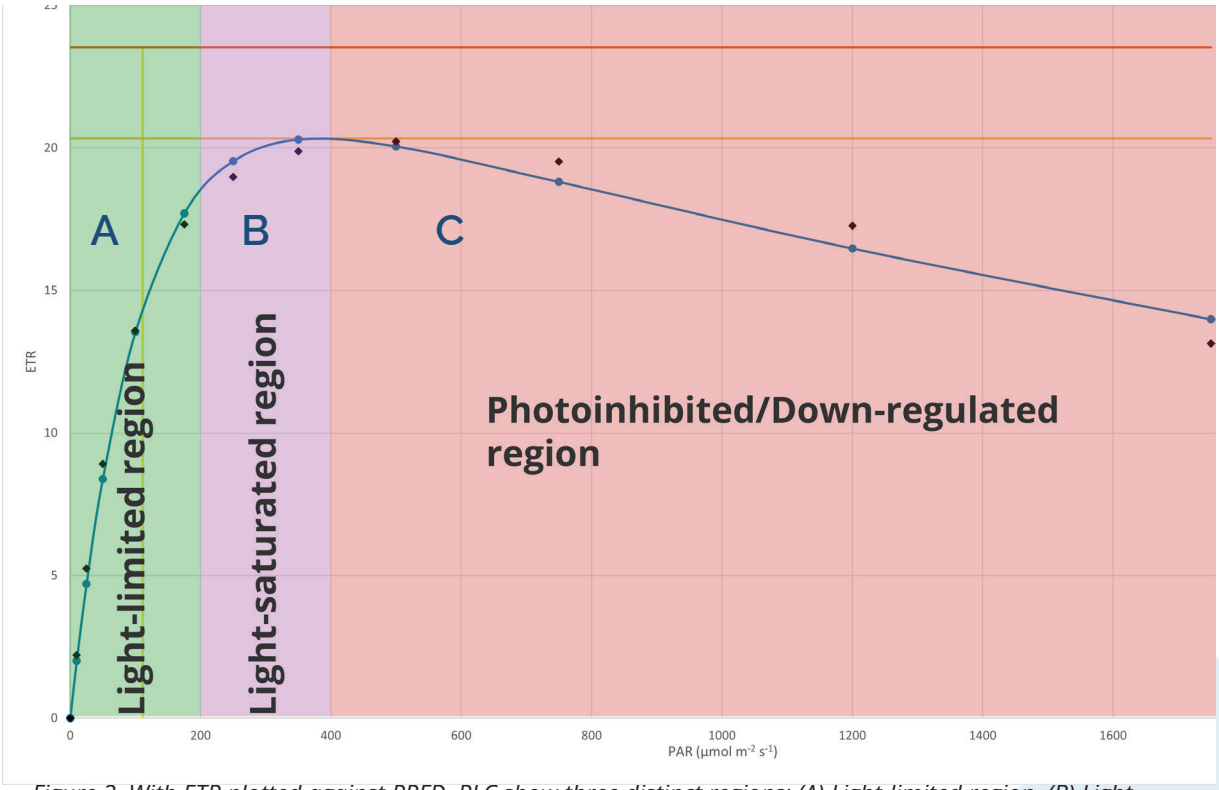

Figure 2. With ETR plotted against PPFD, RLC show three distinct regions; (A) Light-limited region, (B) Light-saturated region and (C) Photoinhibited/Down-regulated region.

Measurement of Rapid Light Curves

RLC protocols typically begin with an initial quasi-dark period of no more than 10 seconds. This allows rapid reoxidation of the primary electron acceptor (Q_A), without substantial relaxation of the non-photochemical quenching coefficient (Schreiber, 2004). This contrasts with an extended, full dark-adaptation period of several minutes or more, used, for example, in measurements of Fast Chlorophyll Fluorescence Induction. Under full dark-adaptation, the light and dark reactions become inactive, leading to complete re-oxidation of the photosystems (Rascher et al., 2000). If complete re-oxidation of PSI and PSII occurred, illumination in the RLC protocol would have an induction effect, making interpretation of the data more complex.

Figure 3. The Handy PEA+ continuous excitation chlorophyll fluorometer.

The quasi-dark period is followed immediately by a saturating pulse. This is followed by a stepped sequence of actinic periods of increasing intensity, each followed by a saturating pulse. A typical duration of the actinic periods is 10 seconds each.

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The ideal settings for the intensities of the actinic steps will generate several data points in each of the three regions described previously. For samples that photosynthesize more efficiently under lower light conditions, it is more beneficial to measure more data points during the light-limited region for a better estimation of the *α* parameter. If the sample is high-light tolerant, then more data points in the light-saturated and photoinhibited regions will provide more interesting results.

Measurement of RLCs using Handy PEA+

The Handy PEA+ (Figure 3) software protocol editor, and specifically the pre-illumination feature (Figure 4), can be used to measure parameters and protocols more typically associated with a non-modulated fluorometer, such as ΦPSII (or Y(II)), ETR, NPQ and quenching analysis models etc.

You can start to make RLC measurements at once using the sample protocol available from the Hansatech Instruments website (Support > Applications). Alternatively, you can install and modify the sample protocol, or create a protocol from scratch.

There is an important caveat about nomenclature. Because PEA+, the control and analysis software used with Handy PEA+, currently assumes that a dark-adapted protocol has been performed, it will by default apply dark-adapted nomenclature to measured parameters in its analysis tools.

Hansatech Instruments have provided a Microsoft Excel® template to perform the graphical and statistical analysis required to interpret RLC data. The nomenclature can be transposed, as per the information in Table 1., after exporting the Handy PEA+ data into the spreadsheet.

Using the sample protocol

1. Download the sample RLC protocol to your PC
2. Connect your Handy PEA+ to the PC
3. Open the PEA+ software on your PC
4. Select the com port on your PC that the Handy PEA is connected to from the Handy PEA > Select Com Port menu option
5. Open the Protocol Editor from the Handy PEA > Protocol Editor menu option
6. In the Protocol Editor, select File > Open from the menu
7. Navigate to the sample protocol file on your PC and click Open
8. On the left side of the Protocol Editor, click the Setup button
9. In the Multi-Recording window (Figure 4), the Illumination settings in the Before Recording area define the actinic light step intensities for the RLC. As discussed previously, the ideal settings for the intensities of theses actinic steps will generate several data points in each of the 3 regions described previously. For samples that photosynthesize more efficiently under lower light conditions, it is more beneficial to define more steps at lower light intensities for a better estimation of the α parameter. If the sample is high-light tolerant, then more actinic steps at higher intensities will provide more interesting results
10. On the left side of the Protocol Editor window, click the Upload button

11. Choose an available protocol slot on the Handy PEA and accept
12. Select and run the protocol from the Handy PEA instrument menu (see "Running the RLC Protocol" below).

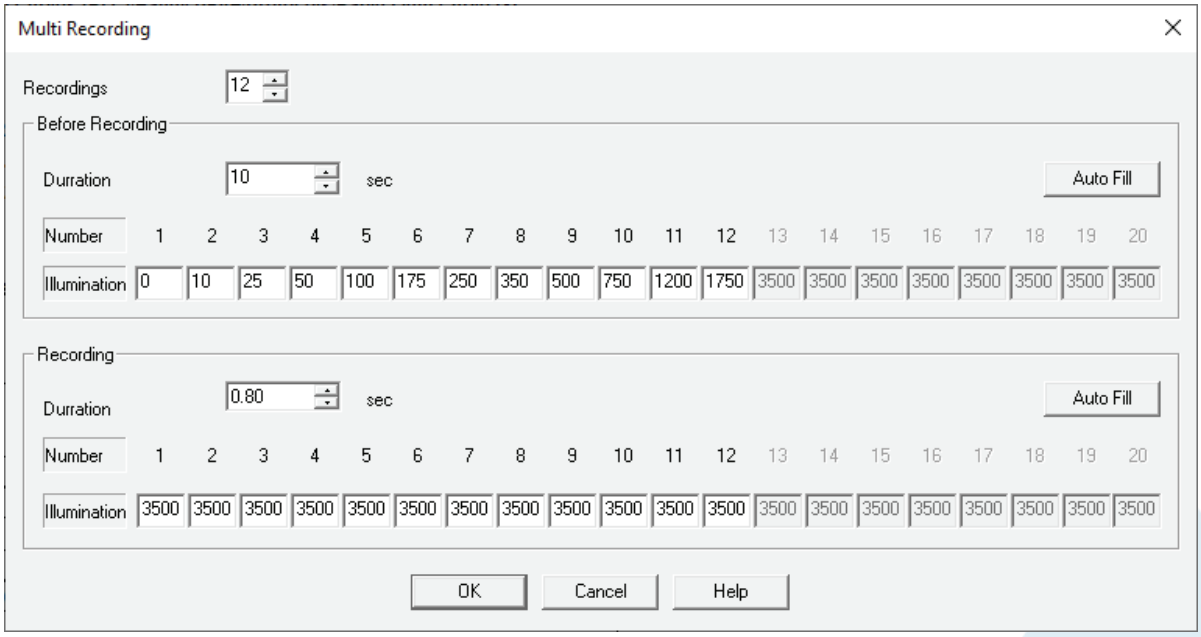

Figure 4. The Multi-Recording window in the PEA+ Protocol Editor showing settings loaded from the sample protocol. The settings in the "Before recording" area of the window define the light intensities of each of the individual actinic light steps.

Defining an RLC Protocol from scratch

Please follow the steps below to define the RLC protocol in the PEA+ software.

1. From the PEA+ software, open the Handy PEA+ protocol editor.
2. Click on the "Title" button and define the name of your protocol.
3. Adjust the gain for your protocol to a suitable level. This level depends on your sample and should be determined either by enabling Autogain in the protocol or by determining the appropriate gain setting before the RLC protocol is created (please refer to the Handy PEA+ manual for further information).
4. From the Control menu on the left of the protocol editor window, toggle the setting to "Multi Recording" and select the number of recordings (in this case, actinic steps) required for the RLC.
5. Set the "Before Recording" duration setting to a period <30 seconds (10 seconds recommended).
6. The quasi-dark period is set by leaving the illumination setting for number 1 as "0".
7. In the subsequent illumination boxes, define a set of actinic illumination intensities to cover all 3 regions mentioned previously. For example, a set of actinic steps such as 10, 25, 50, 100, 175, 250, 350, 500, 750, 1200, 1750 would be sufficient for a low-light/shade plant; for a high-light tolerant plant, the higher light steps in this range may not be supra-saturating and more higher intensities should be selected as opposed to the more numerous lower light intensity steps.
8. Set the recording duration to 1 second.

9. Set all recording illumination settings to >3500 µmol m^{-2} s^{-1} (or the highest your Handy PEA+ will allow).
10. Upload the protocol to the Handy PEA+ control unit.

Running the RLC Protocol

1. On the Handy PEA+ instrument, navigate to the Protocols menu (select System > Protocol or for direct screen access, press 1 > 2 > OK) and select the uploaded protocol to view the settings.
2. Press the Reset key to go to the Main Menu.
3. Disable the measurement Warnings setting by selecting System > Configure > More.. and setting Warnings to "No". Following the initial saturating pulse measurement, some or all subsequent measurements will have inherently low Fm and Fv/Fm values. This is expected, so measurement warnings are not necessary.
4. Ensure all other settings including ID Entry, Gain, Autosave etc. are configured according to your requirements. Please refer to the Handy PEA+ manual for further information.
5. Place a Handy PEA+ dark-adaptation leafclip on the required sample but do not close the shutter since complete re-oxidation of the photosystems will cause the RLC to have an induction effect, making data interpretation more complex (Rascher et al., 2000).
6. Fit the Handy PEA+ sensor to the leafclip and, as quickly as possible to avoid prolonged dark-adaptation of the sample, begin the measurement, ensuring that the sample stays securely in position within the leafclip throughout the duration of the experiment.
7. Once complete, connect the Handy PEA+ to the PC and download the recorded files.

Analysing the RLC Data

As described previously, PEA+ software by default assumes that full dark-adaptation has been performed and applies nomenclature on that basis. The data captured during the RLC protocol is from a light-adapted sample. Therefore, for each of the measurements made during the protocol, the parameters displayed in PEA+ should be re-labelled as shown in Table 1. below.

Default nomenclature in PEA+ software	Nomenclature for parameters measured in RLC protocol
Fo	F
Fm	Fm'
Fv	Fq'
Fv/Fm	ΦPSII

Table 1. Transposition of default nomenclature for RLC parameters made with Handy PEA+

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PEA+ software does not currently offer the graphical and statistical analysis required to interpret RLC data. However, Hansatech Instruments have provided a Microsoft Excel® template into which Handy PEA+ data can be transposed.

The RLC template models a curve using the equation of Platt et al. (1980) from raw data collected with the Handy PEA+. The template requires the Solver Add-in to be enabled within Excel® (Please view this link for further information on how to enable Solver). The Solver add-in is used to calculate the best solution/fit using the GRG nonlinear method minimising the Sum of the Squared estimate of Errors (SSE).

The template includes some sample RLC analysis worksheets for reference (Figure 5).

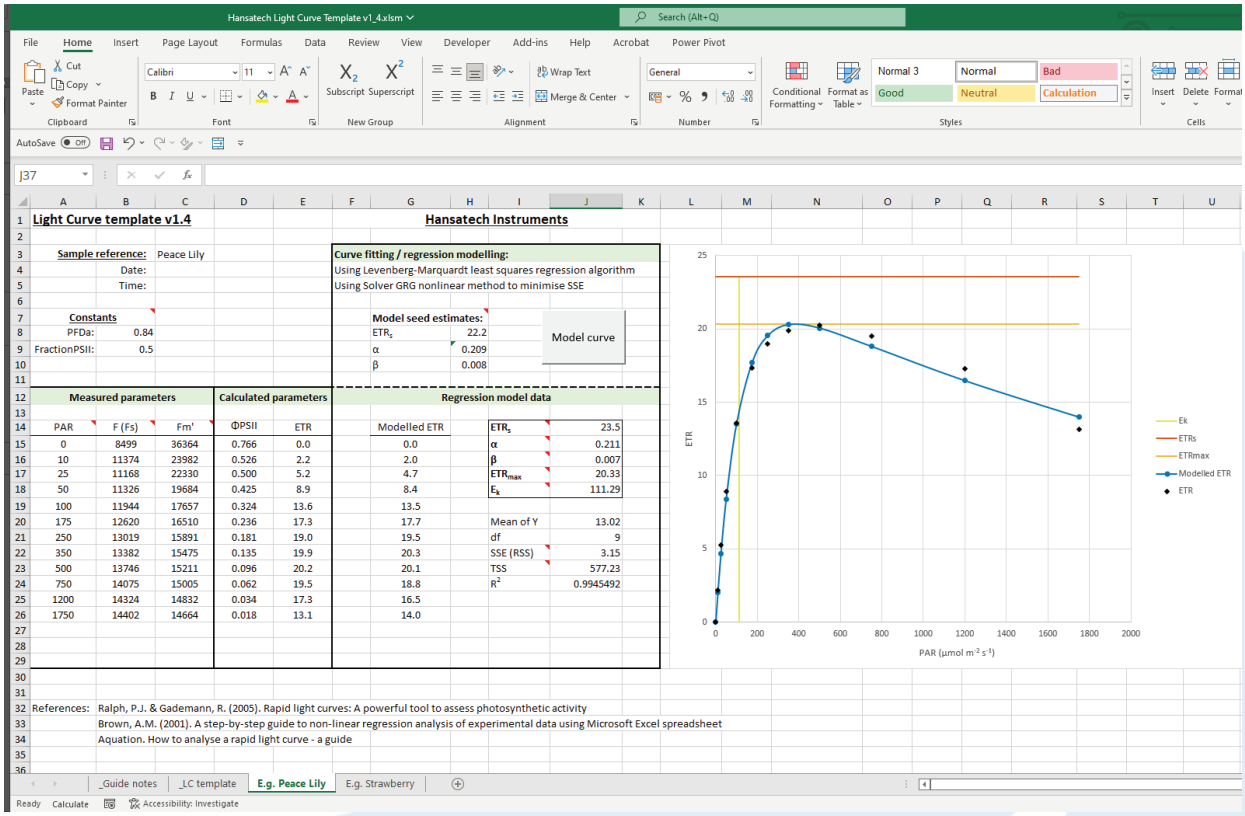

Figure 5. The Rapid Light Curve Excel® template allows data from the Handy PEA+ to be analysed by plotting Electron Transport Rate over PPFD. The Solver add-in is used to calculate the best solution/fit using the GRG nonlinear method minimising the Sum of the Squared estimate of Errors (SSE) and calculates best fit values for ETRs, α, β, ETR_{max} and E_k.

The template can be downloaded from the Support > Applications page of the Hansatech Instruments website. The following guidelines provide a walkthrough of transposing data from PEA+ software into the RLC Excel® template. For specific commands and functionality within PEA+, please refer to the Handy PEA+ manual.

1. Ensure all measured records from the RLC protocol are highlighted in the PEA+ Summary tab.
2. Open the Parameters tab and ensure that the parameters Fo and Fm are displayed. If they are not, they can be enabled from the Tools >'Parameter View' Parameters menu option.

3. Copy the blank RLC template sheet into a new worksheet for each data set you wish to model.
4. Under the "Measured parameters" heading, type the Fo value from the first recording in PEA+ into cell C15 under the heading "F".
5. Type the Fm value from the first recording in PEA+ into cell D15 under the heading " Fm' ".
6. From the Settings tab in PEA+ take the PAR value from the "P. Light" column (should be 0 on the first step) and type it into cell B15 under the "PAR" heading. If the PAR value is not displayed in PEA+, the "P. Light" column is toggled with the "Ip" tickbox on the left-hand side of the window.
7. Values for ΦPSII and ETR are calculated automatically. ETR is calculated using the constants defined in cells B8 and B9. PFD_a and $Fraction_{PSII}$ are set to default values of 0.84 and 0.5 respectively (Baker, 2008) but can be over-ridden if necessary.
8. Enter F, Fm' and PAR values for the remaining measurements rows 16 onwards until all ΦPSII and ETR values have been calculated.
9. Click the "Plot" button to run Solver. This will minimise SSE and a curve will be generated. Best fit values for ETRs, α, β, ETR_{max} and E_k will be calculated.

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Products

Hansatech Instruments product range covers a wide range of applications in the fields of photosynthesis & cellular respiration. We manufacture oxygen measurement systems based on Clark type polarographic oxygen sensors, chlorophyll fluorescence measurement systems for both continuous excitation & pulse-modulated measurement techniques & optical instrumentation for the measurement of sample chlorophyll content.

Support

Purchasers of Hansatech Instruments products can be assured of ongoing support & prompt & efficient attention to enquiries at all times. Customers are encouraged to register their instruments on our website which allows access to our Support Ticketing System in addition to instruments manuals & software upgrades

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