Handy PEA+

Advanced continuous excitation chlorophyll fluorimeter

Hansatech Instruments

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- Compact (170 x 85 x 40mm), lightweight (565gms)
- USB2.0 Communications
- Large-scale screening capacity up to 1000 full trace data files
- High time resolution detection for discrimination of fast chlorophyll fluorescence induction kinetics
- Full suite of OJIP analysis parameters (Strasser *et al 2000)*
- Saturating high-intensity focused LED array for accurate determination of Fm
- Upload user-defined, repeatable protocols for automatic field execution
- Interchangeable sensor unit cables with lengths of up to 10 metres
- Powerful Windows® data transfer & analysis software included

Handy PEA+ chlorophyll fluorimeter

Handy PEA+ consists of a compact, light-weight control unit encapsulating sophisticated electronics. This provides the high-time resolution which is essential in performing measurements of fast chlorophyll fluorescence induction kinetics.

Simple to configure and operate, basic measurement functionality can be defined directly on the Handy PEA+. More complex experimental design may be acheived using the protocols feature which allows up to 5 user-defined protocols to be stored in memory for different field applications. Protocols are written using a custom Windows® software package, PEA+ (supplied). A tactile keypad allows selections and inputs to be made and a liquid crystal display module presents menu options and data.

The sensor unit consists of an array of 3 ultra-bright red LED's which are optically filtered to a peak wavelength of 650 nm (which is readily absorbed by chlorophyll) at a maximum intensity of up to 3500 µmol m^{-2} s^{-1} at the sample surface. The LED's are focused via lenses onto the leaf surface to provide uniform illumination over the area of leaf exposed by the leafclip (4mm dia). An optical feedback circuit monitors and corrects changes in the output intensity of the LED's which can be caused by internal heat build-up within the LED's themselves. The circuit also compensates for intensity changes caused by variation in ambient temperature.

The sensor unit is fitted with a high-performance pin photodiode. Optical design and filtering ensure that it responds maximally to the longer wavelength fluorescence signal and blocks the reflected shorter wavelength LED light used as the source of illumination. Variable rate analysis allows fluorescence signals to be sampled at different acquisition rates throughout the different phases of the induction kinetic. Initially, data is sampled at 10 µsecond intervals for the first 300 µseconds with subsequent induction phases analysed at lower acquisition intervals as the rate of kinetic activity reduces.

Up to 1000 recordings of between 0.1 – 300 seconds may be saved in the onboard memory of the Handy PEA+. Calculated parameters may be viewed onscreen with more comprehensive data display acheived by transferring saved data via USB to a PC where the supplied PEA+ Windows software allows a variety of numerical and graphical presentation options.

Leafclips and sample dark adaptation

Continuous excitation fluorescence systems rely on the use of a suitable leafclip system with 2 functions. Firstly, the leafclip sheilds the fluorescence detector from ambient light which would otherwise "blind" the sensor due to the comparatively high levels of red/infra-red light within the same waveband as fluorescence itself. Secondly, the leafclip pre-conditions or dark adapts a section of the sample prior to the measurement.

Any measurement of the maximum photochemical efficiency of Photosystem II (Fv/Fm) requires the sample to be fully dark adapted prior to measurement. During dark adaptation, all reaction centres within the sample are fully oxidised making them available for photochemistry and any latent chlorophyll fluorescence yield is quenched. This process takes a variable amount of time and depends upon plant species, light history prior to the dark transition and whether or not the plant is stressed. Typically, 15 – 20 minutes may be required to dark adapt effectively.

Handy PEA+ leafclips are constructed from white plastic making them small and lightweight. The locating ring (which interfaces with the Handy PEA+ sensor) is positioned over the required area of sample and has a central 4mm diameter hole which is covered using a shutter plate. During measurement, this shutter slides back to expose the dark adapted sample to the focussed LED's and fluorescence detector. A silvered underside reflects incident light minimising the build-up of heat on the sample and ensuring that the measurement is unaffected when measuring in high ambient light conditions.

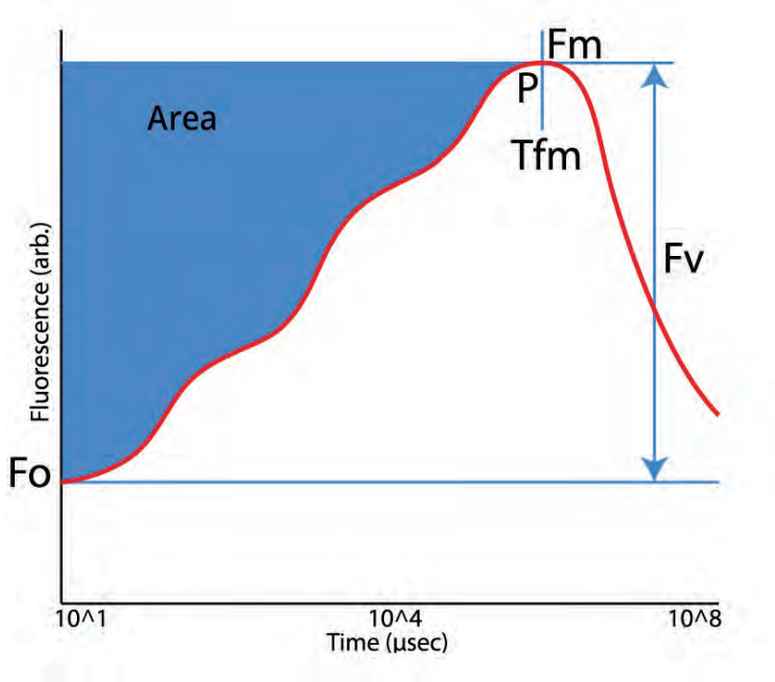
Parameters measured

Common parameters

Fo - Represents emission by excited chlorophyll *a* molecules in the antennae structure of Photosystem II. The true Fo level is only observed when the first stable electron acceptor of Photosystem II called Qa is fully oxidised. This requires thorough dark adaptation.

Fm - The maximum fluorescence value obtained for a continuous light intensity. This parameter may only be termed as maximal if the light intensity used is fully saturating and the electron acceptor Qa is fully reduced.

Fv - Indicates the variable component of the recording and relates to the maximum capacity for photochemical quenching. Calculated by subtracting the Fo value from the Fm value.



Fv/Fm - An indication of the maximum quantum efficiency of Photosystem II and widely considered to be a sensitive indication of plant photosynthetic performance.

Presented as a ratio between 0 and 1, healthy samples typically achieve a maximum Fv/Fm value of approx. 0.85. Values lower than this will be observed if a sample has been exposed to some type of biotic or abiotic stress factor which has reduced the capacity for photochemical quenching within PSII. Fv/Fm is presented as a ratio of variable fluorescence (Fv) over the maximum fluorescence value (Fm).

Tfm - Indicates the time at which the maximum fluorescence value (Fm) was reached. May be used to indicate sample stress which causes the Fm to be reached much earlier than expected.

Area - The area above the fluorescence curve between Fo and Fm is proportional to the pool size of the electron acceptors Qa on the reducing side of Photosystem II. If electron transfer from the reaction centres to the quinone pool is blocked (such as is the mode of action of the photosynthetically active herbicide DCMU), the area will be dramatically reduced.

OJIP analysis

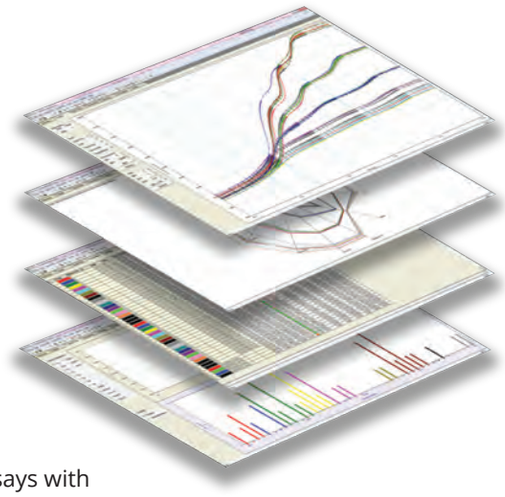
PEA+ software extracts fluorescence values from the recorded data at 5 pre-defined Time Marks. The times are:

> T1 = 50 microseconds
> T2 = 100 microseconds
> T3 = (K step) 300 microseconds
> T4 = (J step) 2 milliseconds
> T5 = (I step) 3 milliseconds

Chlorophyll fluorescence values at these Time Marks are used in conjunction with other measured and calculated values to derive a series of further biophysical parameters, all referring to time base 0 (onset of fluorescence induction).

These parameters quantify the photosystem II behaviour for specific energy fluxes (per reaction centre) for absorption, trapping, dissipation and electron transport in addition to the maximum yield of primary photochemistry, the efficiency with which a trapped exciton can move an electron into the electron transport chain further than QA- and the quantum yield of electron transport.

PEA+ software



PEA+ is a multi-function Windows® program supplied with Handy PEA+ for system configuration, data acquisition and post-measurement analysis.

Several different data presentation techniques have been combined in order to effectively demonstrate subtle differences in the fluorescence signature of samples which could be indicative of stress factors affecting the photosynthetic efficiency of the plant. Data may be presented in graphical, tabulated or radial plots which can all be tailored to display any number of the 58 parameters measured by Handy PEA+. Transferred data may be exported to CSV format for further statistical analysis in external software packages.

PEA+ allows emhanced configuration of the Handy PEA via the Protocol Editor feature. Protocols may be defined to include single or multiple measurement assays with optional pre-illumination periods which can then be uploaded to the memory of Handy PEA+ via USB communications. The use of protocols ensures maximum reproducibility of results during field applications involving large-scale screening away from a laboratory environment.

PEA+ will run on all supported Microsoft operating systems.

System components

Handy PEA+ systems are supplied with the following components

- Handy PEA+ control unit and sensor
- HPEA/LC x 2: (20 Leafclips)
- Mains powered battery charger
- Protective carry bag
- USB data transfer cable
- USB Drive containing PEA+ software and manuals.

Related systems

Pocket PEA is designed to be a powerful, effective screening tool capable of performing single flash measurements. Configuration is limited compared with Handy PEA+ with the only configurable options being measurement duration (1, 3 or 10 seconds) and light intensity (up to 3500 µmol m^{-2} s^{-1}). M-PEA is a more advanced fluorescence system with the added functionality of P700+ aborbance and delayed fluorescence capability.

Technical specifications

Handy PEA Fluorimeter

Dimensions:	170 (l) x 85 (w) x 40mm (d). Weight: 565g
Communications:	USB2.0
Operating Conditions:	0 - 40°C. Non-condensing humidity
Battery:	3 x rechargeable Ni-MH 3.6V, 1.8Ahr
Battery Charger:	Integral switch mode charger 8-13.5V input (nominal 12V input)
Display:	8 line x 20 character LCD display
Illumination:	Focused array of ultra-bright red LED's with NIR short pass cut-off filters. Peak wavelength 650 nm. Spectral-line half width 22 nm.
Max. intensity at sample:	Up to 3500 µmol m^{-2} s^{-1}
Detector:	Fast response PIN photodiode with RG9 long pass filter
Electronics:	16-bit microprocessor. 12-bit resolution. A/D 10µsec acquisition rate. 8-bit DAC for light control. Real time clock
Record Length:	0.1 - 300 seconds
Memory:	512K battery backed RAM. (Up to 1000 one second duration recordings with full trace data)
Leafclips:	20 x injection moulded clip system with silvered locating ring, 4mm sample aperture and sliding shutter blade.

Parameters measured

OJIP Data:
tFm, Area, Fo, Fm, Fv

Normalised data:
Fo/Fm, Fv/Fm, Fv/fo, Vj = (Fj-Fo)/(Fm-Fo), Vi = (Fi-Fo)/(Fm-Fo)

Specific fluxes:
ABS/RC, DIo/RC, TRo/RC, ETo/RC, REo/RC

Apparent fluxes per CSo:
ABS/RC, DIo/RC, TRo/RC, ETo/RC, REo/RC

Partial performances:
Γ(RC)/(1-Γ(RC)), Φ(Po)/(1-Φ(Po)), Ψ(Eo)/(1-Ψ(Eo)), PI(abs), Δ(Ro)/(1-Δ(Ro))

Time marks:
Ft1, Ft2, Ft3, Ft4, Ft5

Partial areas:
Fo to Ft1, Ft1 to Ft3, Ft1 to Ft4, Ft1 to Ft5, Ft3 to Ft4, Ft4 to Ft5, Ft5 to Fm

Slopes & integrals:
dVg/dto, dV/dto, Sm = Area/Fv, N = Sm/Ss, Sm/tFm

Yield = flux ratios:
TRo/ABS = Φ(Po), ETo/TRo = Ψ(Eo), ETo/ABS = Φ(Eo), REo/ETo = Δ(Ro), REo/ABS = Φ(Ro)

Apparent fluxes per CSm:
(ABS/CSm)~Fm, DIo/CSm, TRo/CSm, ETo/CSm, REo/CSm

Total performance, driving force & rates:
PI(total), DF(abs), DF(total), kP/ABS * kF, kN/ABS * kF

User parameter:
3 User-entered values

Hansatech Instruments is a British company that has been developing high quality scientific instrumentation for over 40 years. Our systems are used widely for teaching & research in cellular respiration & photosynthesis programs in more than 100 countries throughout the world. We have gained an enviable reputation for quality, reliability & excellent price/performance.

Our product range consists of a range of modular solutions for the measurement of oxygen using Clark type polarographic sensors. We also develop chlorophyll fluorescence measurement systems using both continuous excitation & pulse-modulated measurement techniques with further optical instrumentation for the measurement of sample chlorophyll content.

Purchasers of Hansatech Instruments products can be assured of ongoing support & prompt & efficient attention to enquiries at all times. Support is available both directly & from our global distributor network. 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.

Hansatech Instruments Ltd
Narborough Road, Pentney, King's Lynn, Norfolk PE32 1JL, UK
Tel: +44 (0)1760 338877 Fax: +44 (0)1760 337303
info@hansatech-instruments.com
www.hansatech-instruments.com

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