M-PEA

Multi-function plant efficiency analyser

Hansatech Instruments

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Multi-function plant efficiency analyser

- Advanced lab and field-based system for investigation of plant photosynthetic efficiency
- M-PEA-1 variant for prompt fluorescence & P700+ modulated absorbance measurements
- M-PEA-2 variant as M-PEA-1 with additional measurements of Delayed Fluorescence (DF) & leaf absorptivity
- Sophisticated sensor unit with all optical emitters & detectors in a robust, enclosed housing
- USB connection to a Windows® PC
- Comprehensive Windows® experimental design, data transfer & analysis software

M-PEA multi-function plant efficiency analyser

The M-PEA (Multi-Function Plant Efficiency Analyser) combines high-quality fast fluorescence kinetic and P700+ absorbance studies with ground-breaking Delayed Fluorescence (DF) measurements providing one of the most comprehensive systems for the investigation of plant photosynthetic efficiency available.

The M-PEA is a laboratory-based measurement system consisting of a control unit and sophisticated, robust sensor unit housing all optical emitters and detectors for all measurement elements.

The system is controlled from a comprehensive Windows® software package (M-PEA+) which allows complex experiments to be designed, uploaded and executed by the M-PEA hardware. Recorded data is quickly downloaded to the software via a USB 2.0 connection.

The control unit is of convenient size with minimal footprint allowing measurements to be made in a busy lab environment where bench space is critical. The front panel consists of a power switch and indicator LED, optical sensor connection and a 4 line LCD display. The rear panel provides input for a 12V DC power supply and a USB2.0 connection socket for interface to the M-PEA+ software running on a Windows® PC.

M-PEA optical sensor

The optical sensor unit is a robust enclosure designed to incorporate sophisticated electronics which effectively controls all of the light sources and detectors. The M-PEA-1 sensor unit includes a high intensity red actinic source, a far-red light source, the prompt fluorescence detector and the modulated emitter/detector pair for P700+ absorbance measurements. M-PEA-2 additionally includes a high sensitivity delayed fluorescence detector and a detector to measure leaf absorptivity.

All the optics are located behind a quartz window which seals the sensor unit providing effective protection for the optical assemblies against dust, dirt and moisture.

M-PEA+ software

M-PEA Plus is a custom Windows® software package created for experimental design and deployment and comprehensive analysis of recorded data.

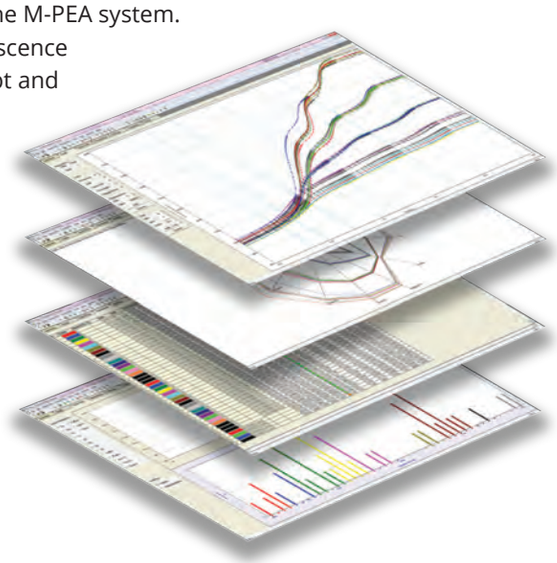
M-PEA+ consists of 2 main elements:

M-PEA+ protocol editor

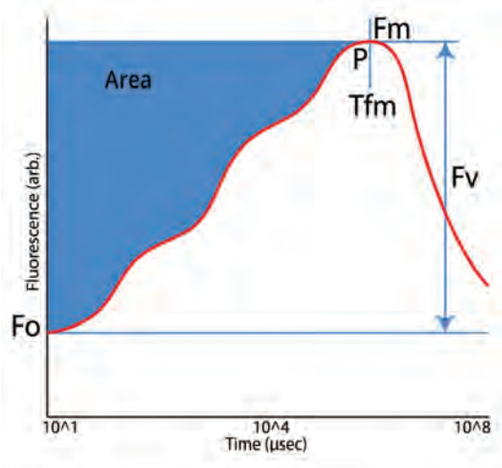
The protocol editor allows the creation of experiments for deployment on the M-PEA system. Experiments can range in complexity from a simple 1 second prompt fluorescence measurement through to repeating, multi-flash measurements using prompt and delayed fluorescence, P700+ and relative absorptivity to probe the activity of PSI and PSII complexes within the photosynthetic apparatus.

Data analysis modules

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



Common parameters measured



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.

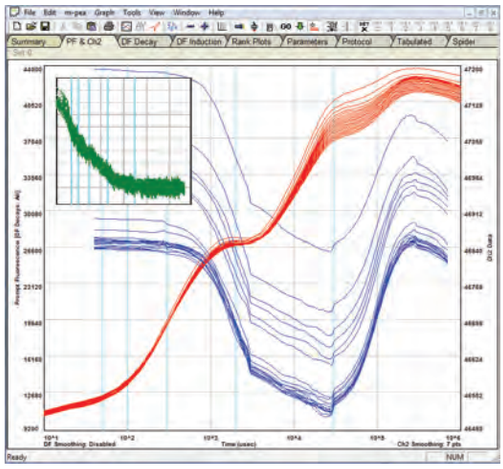
Fv/Fm - An indication of the maximum quantum efficiency of Photosystem II and widely considered to be a sensitive indication of plant photosynthetic performance.

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.

P700 absorbance



The photosynthetic electron transport chain consists of 3 large protein complexes Photosystem II (PSII), Cytochrome (cyt b6/f), Photosystem I (PSI). P700 is the term used to describe the chlorophyll within the reaction center of PSI as this is the wavelength of light to which the photosystem is most reactive. Upon illumination using a strong light source, the photosynthetic electron transport chain is almost entirely reduced.

The electrons from this reduction in turn activate the enzyme ferredoxin-NADP+ reductase which leads eventually to NADP reduction and CO_2 fixation. This initial reduction process is represented by the OJIP steps of the Kautsky induction curve during prompt fluorescence measurements.

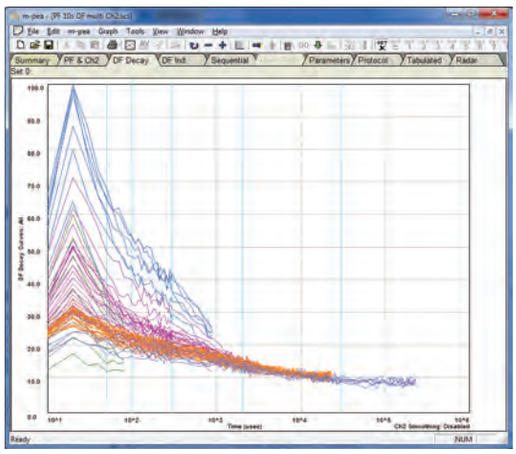
The oxidation of P700 causes an increase in absorbance at wavelengths falling in the 800 – 850nm band. M-PEA measures the transmission of P700 using a modulated LED with a peak wavelength of 820nm and a highly sensitive photodiode to monitor the absorbance of the PSI complex during prompt fluorescence measurements.

Since the 820nm LED is not actinic, M-PEA is able to use high light intensities without disturbing the PSII complex. Therefore, M-PEA presents a convenient, reliable method of measuring chlorophyll a fluorescence and transmission at 820nm simultaneously, thus allowing the study of the electron transport process during the Kautsky induction at both end of the photosynthetic electron transport system.

M-PEA is also fitted with a far-red light source which can be used to preferentially excite the PSI complex. Re-reduction occurs via the intersystem electron transport chain by PSII activity, with electrons originating from hydrolysis.

The M-PEA uses an optically filtered, modulated 820nm LED for high quality P700+ absorbance measurements. P700+ activity is recorded using an optimised low noise, fast response PIN photodiode and 16-bit A/D converter providing an excellent signal-to-noise ratio.

Delayed fluorescence measurements



Delayed fluorescence (DF) has much in common with prompt fluorescence (PF) because it originates from the same chlorophyll molecules of the Photosystem II antenna complexes.

DF is essentially light that is emitted from green plants, algae and photosynthesising bacteria for a short time after they have been exposed to light, but after the prompt fluorescence emission has decayed. Delayed fluorescence occurs in the red/infra-red region of the spectrum (the same as prompt chlorophyll fluorescence). However, the intensity of the delayed fluorescence emission is lower than that of prompt fluorescence by at least two orders of magnitude therefore requiring extremely high-sensitivity apparatus to measure the signal.

Like PF, the properties of the DF emission are highly sensitive to the functional state of Photosystem II and the photosynthetic reaction chain as a whole. Theoretically, DF bears even more information about the photosynthetic processes than PF. Still, a fluorescence measuring instrument can be found in almost every plant science research laboratory, while DF has not gained much popularity as a practical method to study photosynthetic organisms. One reason for such injustice is that DF is harder to register than PF. But the greatest difficulty in using DF is its interpretation, or extracting the valuable information from this extremely complex signal.

Fortunately, in recent years we've witnessed major advances both in development of electronic engineering and also in the theory of DF measurements. We are more and more able to utilise DF for practical scientific research. It is the combination of these 2 factors that has lead to the development of M-PEA.

The delayed fluorescence emission, natural to all green plants, has been known to scientists for over fifty years. It was first discovered by Strehler and Arnold (1951) when they were attempting to use firefly luminescence for the measurement of the light-induced accumulation of ATP in the green alga Chlorella. They found that even without the addition of luciferase and luciferin, there was a long-lived glow from algal cells and chloroplasts in darkness following illumination. The observed delayed fluorescence was characteristic of different photosynthesising samples used—leaves (Strehler and Arnold 1951), chloroplasts and photosynthesising bacteria (Arnold and Thompson 1956). Strehler and Arnold postulated that it was in fact chemiluminescence of the chlorophyll, caused by reversal of the photosynthetic reactions. The close relationship between DF and the photosynthetic reactions was confirmed undoubtedly in many studies and sometimes DF was found even more sensitive than the prompt fluorescence (Kramer and Crofts, 1996).

M-PEA variant feature comparison

	Signals measured			
	Prompt fluorescence	P700+ absorbance	Delayed fluoresence	Relative absorptivity
M-PEA-1	✔	✔	✘	✘
M-PEA-2	✔	✔	✔	✔

System components

Pocket PEA+ systems are supplied with the following components

- M-PEA control unit and sensor
- HPEA/LC x 2: (20 Leafclips)
- Mains powersupply
- Transit case
- USB PC connection cable
- USB Drive containing M-PEA+ software and manuals.

Technical specifications

M-PEA control unit

Electronics: 1 x high performance 16-bit microcontroller, 1 x enhanced flash 8-bit controller, Dual channels: 1 x modulated, 1 x non-modulated, 16-bit resolution A/D 10µs acquisition rate. Dual 16-bit D/A light source, controller
Memory: 32 Mb internal memory storage
Display: 4 line x 20 character LCD
Recording: Duration 0.001 - 300 seconds (repeatable up to 100 x per protocol)
Comms: USB2.0 full speed (12 Mb/s)
Power: 12V @ 1A DC
Dimensions: 230 (w) x 190 (d) x 85mm (h). 1.4kg

M-PEA-1 optical sensor unit

Illumination: Actinic: Focused ultra-bright LED with NIR short pass cut-off filter. Dominant λ625nm. Spectral half-width 20nm. Max intensity 5000 $\mu molm^{-2}s^{-1}$. Far-red: Focused ultra-bright LED with long pass filter. Max intensity >1000 $\mu mol\ m^{-2}\ s^{-1}$
P700+: Optically filtered pulse modulated 820nm LED. Intensity 0 - 100% in 1% steps.
Detectors: PF: Low noise, fast response PIN photodiode with 730nm (± 15nm) bandpass filter.
P700+: Low noise, fast response PIN photodiode with optical bandpass filter.

M-PEA-2 optical sensor unit

Illumination: Sources As in M-PEA-1 sensor unit
Detectors: As M-PEA-1 but with additional detectors:
Delayed fluorescence: High sensitivity wideband avalanche photodiode with 7 30nm (± 15nm) bandpass filter.
Leaf aborptivity: Low noise, fast response PIN photodiode

Prompt fluorescence 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.

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