FMS-300

Pulse-modulated chlorophyll fluorometer for teaching & research

Document version: 2.00
Date: 19th February 2024

Hansatech
Instruments

Key features

> Versatile functionality:

Suitable for both teaching and research applications. FMS-300 can demonstrate complex concepts, conduct experiments, collect data, analyse results, and facilitate collaborative learning or research projects.

> Fast & slow fluorescence data:

Presents fast and slow fluorescence data with equal emphasis. Fast fluorescence kinetics are captured during every saturating pulse for both dark and light-adapted sample states, presenting opportunities for novel research.

> Comprehensive parameter display:

Parameter sets relevant to the selected routine are calculated for each saturating pulse. For dark-adapted samples, OJIP parameters in addition to parameters such as Fv/Fm are calculated. Light-adapted parameters including ETR, ΦPSII, Lake and Puddle models for non-photochemical quenching, and both a calculated Fo' and Fo' measured under far-red light are calculated where appropriate.

> Developed for high performance:

A measuring pulse width of 400 ns from a dedicated LED allows greater intensities to be implemented yielding a strong, low-noise signal. Measuring pulse intensity can be defined between *0.001 - 0.1 µmol m^{-2} s^{-1}. Actinic and saturating pulse intensities of up to 3,000 and **20,000 µmol m^{-2} s^{-1} respectively can be delivered at the sample surface.

> Streamlined data capture:

FluoroControl software provides 6 pre-set experimental routines, which can be further configured, if required. This enables rapid learning and efficient, hassle-free data acquisition.

> Intuitive software with simplified control set:

Developed from the ground up with simplicity in mind. A user-friendly interface with minimal learning curve allows easy navigation of the different functions and options.

> Data visualisation & analysis:

The measuring LED performance means that 100% raw data is presented with no requirement to damp or average. Robust data visualisation, analysis and export tools allow users to interpret and present findings effectively. Valuable for both teaching complex concepts and conducting in-depth analysis in research applications.

> Measuring light & actinic/saturating LED colours:

4 variants with different pairings of LED colour. Supplied with either blue/blue (455 nm), red/red (624 nm), blue/white or red/white measuring/actinic and saturating LEDs. All variants contain a far-red LED (730 nm) for determination of Fo' during the quenching analysis routine.

** Depending on optical accessory in use. Up to 0.1 µmol m^{-2} s^{-1} when using dark-adaptation or PTL-100 leafclips. Up to 0.41 µmol m^{-2} s^{-1} when using FMS/LG3 tapered light guide.*

*** Depending on optical accessory in use. Up to 60,000 µmol m^{-2} s^{-1} when using FMS/LG2 tapered light guide and up to 90,000 µmol m^{-2} s^{-1} when using FMS/LG3 tapered light guide.*

Welcome to the FMS-300

FMS-300 is a state-of-the-art, Pulse Amplitude Modulated (PAM) chlorophyll fluorometer combining the usability of a teaching system with the power and functionality to provide high-level research-grade data. A wide range of accessory components allow experiments to be conducted on many different sample types.

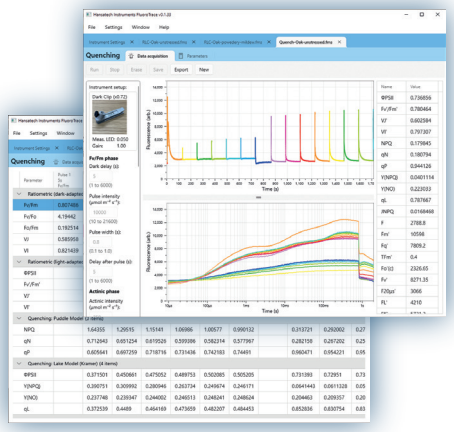
Newcomers to the technique are quickly able to acquire and analyse data, making FMS-300 an ideal system for teaching chlorophyll fluorescence. Yet it is also a highly capable research instrument offering flexibility, functionality, and data acquisition of exceptional quality. Extensive features and capabilities allow the system to be used to demonstrate complex concepts, conduct experiments, collect data, analyse results, and facilitate collaborative learning or research projects.

Primarily a laboratory-based system, FMS-300 can extend to greenhouse and field applications when coupled with an appropriate portable power source.

The enviable signal quality is achieved via ultra-short measuring pulses with a standard frequency of 10 Hz (up to 100 kHz during fast fluorescence capture). At just 400 ns per pulse, FMS-300 can emit high-intensity measuring pulses with user-defined average intensities up to 0.1 µmol m^{-2} s^{-1}.

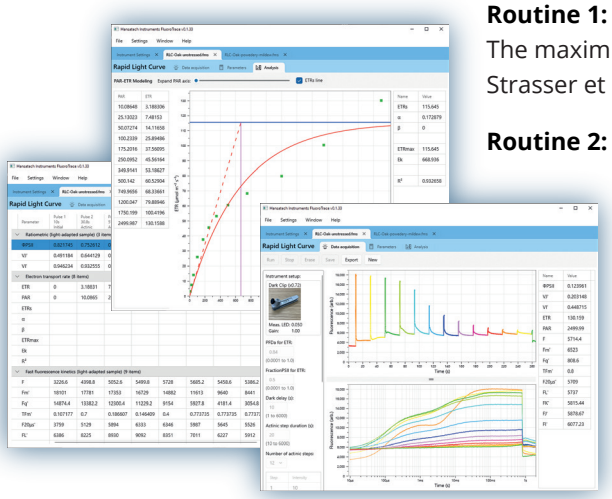
This combination of high-intensity and ultra-short pulse width produces a strong fluorescence signal with no requirement for signal damping or data averaging. The user is presented with 100% raw instrument data.

FluoroControl software



FMS-300 is supplied with a sophisticated, yet user-friendly software package. FluoroControl controls all aspects of instrument operation and has been developed with simplicity of use at its core. An intuitive, tab-based interface ensures a shallow learning curve allowing users to begin measurements quickly. 6 configurable measurement routines, which cover all conventional fluorescence protocols, are used to acquire data. A comprehensive set of measured and calculated parameters are presented for every saturating pulse event with fast fluorescence (OJIP) data captured in both light- and dark-adapted states. Measured data can be easily exported to CSV format for further analysis in external software.

Experimental routines



Routine 1: Maximum Quantum Yield (Fv/Fm and OJIP)
The maximum quantum yield of PSII (Fv/Fm), and a full suite of OJIP (JIP-Test, Strasser et al., 2004) analysis parameters.

Routine 2: Effective Quantum Yield (ΦPSII, ETR)
The operating efficiency of PSII (ΦPSII or Y[II]). Used in the calculation of linear electron transport through PSII (ETR).

Routine 3: Quenching
Based on the protocol defined in Baker et al. (2004). Puddle and Lake models (Kramer 2004, Hendrickson 2004) of NPQ are provided with fast fluorescence data at every saturating pulse event.

Routine 4: Light Response Curve
Electron transport rate (ETR) for periods of increasing actinic intensity. ETR vs PAR plotted with ETRmax, Ek, α, and β parameters calculated. Allows both rapid and steady-state light curves to be executed.

Routines 5 and 6: Multi- Maximum and Effective Quantum Yields
Allows successive measurements of dark-adapted Fv/Fm / OJIP and ΦPSII / ETR measurements respectively to be made. Facilitates comparison of several different samples, replication or screening of, for example, multiple phenotypes with all measurements over-plotted on the same graph.

Leafclips

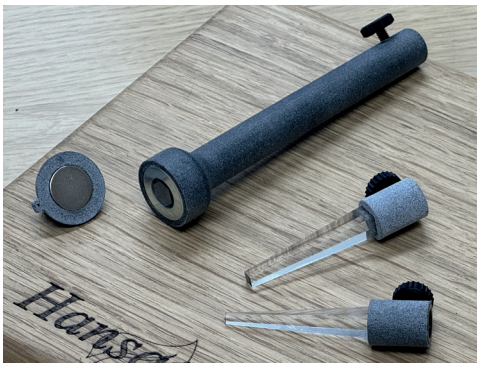
FMS-300 is supplied with a set of 10 dark-adaptation leafclips (DLCs) to be used in experiments where a dark-adapted sample is required before or during the experiment. DLCs can be used for a wide variety of different samples such as broad-leaf species (including some larger Arabidopsis leaves), grasses and needle species. For faster throughput, multiple DLCs can be used to dark-adapt a number of samples at the same time in advance of making measurements.

An open-faced leafclip for measurement of samples under ambient light conditions is supplied with FMS-300 as standard. The leafclip has a front-mounted PAR sensor and a leaf temperature sensor mounted within the underside of the clip. The fibre-optic is positioned at 60° to the plane of the sample and in close proximity to it. This maximises both sample illumination intensity and detection of the fluorescence signal.

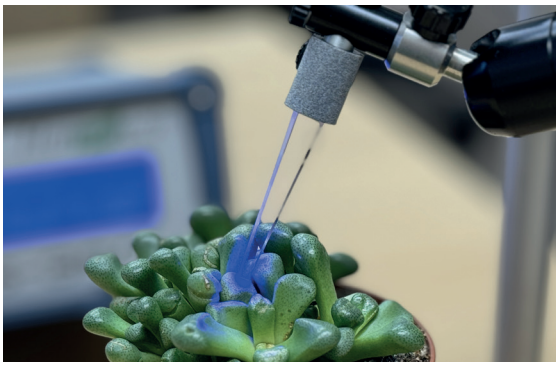
FMS-300 is also supplied as standard with a Manfrotto articulated variable friction arm and clamp to support the fibre-optic cable and leafclips. This attaches to a retort stand and provides a convenient method of securely holding the fibre-optic cable and leafclips in place during experiments.

Optional accessories

A range of accessory components extend the functionality of FMS-300 for different sample types. Hansatech Instruments offer 3 different light guides made from optical grade, highly polished borosilicate glass. These connect to the fibre-optic cable in place of a standard leafclip, to allow measurements in a wide range of specialised applications.



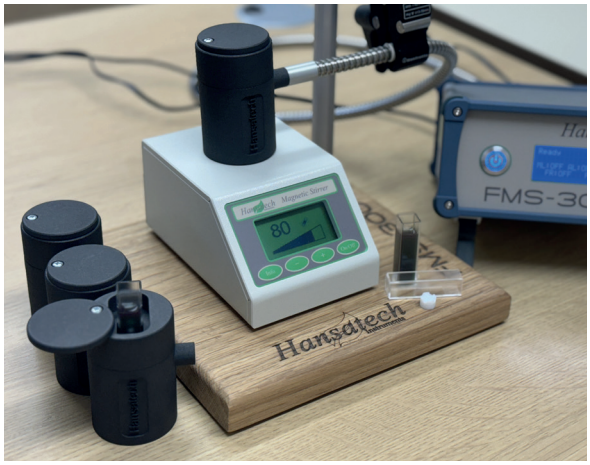
The 8 x 100mm hexagonal light guide is suited to e.g. cacti and succulents or insertion into water tanks for measurements on e.g. sea grass or coral. The light guide is protected by a sleeve and laminar samples are held in place with a magnetic puck.



For measuring smaller samples such as Arabidopsis, needles and lichens, two tapered light guides are available. The tapered form of these light guides focuses the light from FMS-300 and increases the maximum saturating pulse intensities. The two light guides have dimensions at the sample end of 4 x 4 mm and 2.5 x 2.5 mm providing up to 60,000 and 90,000 µmol m^{-2} s^{-1} respectively.

For measurements of liquid samples such as algae, a chamber designed to accept 4.5 ml spectrophotometer cuvettes is available. Liquid sample chambers are fitted with a swivel-action lid to enable samples within the chamber to be dark-adapted.

A lateral optical port allows the FMS-300 fibre-optic cable to be inserted into the chamber so that the tip of the fibre-optic is in direct contact with the outer wall of the sample cuvette. Samples with a minimum optical density (O.D.) of 0.1 can be measured with both slow and fast fluorescence kinetics presented following every saturating pulse event. Using multiple chambers means that multiple samples can be dark-adapted simultaneously prior to measurement. Sample chambers are compatible with the Hansatech Instruments magnetic stirrer or existing magnetic stirrer plates so that samples can be mixed during the experiment.



To complement the supplied fibre-optic support arm, a solid oak-base retort stand with laser-etched Hansatech Instruments and FMS-300 badging is available.

A choice of four LED colour options

The maximum intensity of the measuring LED in the B and BW variants is up to 0.1 µmol m^{-2} s^{-1}. In the R and RW variants, maximum intensity of the measuring LED is up to 0.058 µmol m^{-2} s^{-1}. Whilst all variants offer exceptional signal to noise qualities, for measurements on most eukaryotic algae or higher plants, the FMS-300-B and BW variants provide the strongest fluorescence response. It is important to understand the absorption characteristics and photochemical requirements of the intended sample when choosing the most suitable FMS-300 variant.

<table>
<tr><th>Variant</th><th>Measuring LED</th><th>Actinic & Saturating LED</th><th>Far-red LED</th><th>Fluorescence Detector</th></tr>
<tr><td>FMS-300-B</td><td colspan="2">Blue – 455 nm (FWHM 27 nm)</td><td rowspan="4">730 nm (FWHM 20 nm)</td><td>> 645 nm</td></tr>
<tr><td>FMS-300-R</td><td colspan="2">Red – 624 nm (FWHM 18 nm)</td><td rowspan="3">> 680 nm</td></tr>
<tr><td>FMS-300-BW</td><td>Blue – 455 nm (FWHM 27 nm)</td><td rowspan="2">Ultra-white</td></tr>
<tr><td>FMS-300-RW</td><td>Red – 624 nm (FWHM 18 nm)</td></tr>
</table>

Table 1: LED configurations for each of the four FMS-300 variants.

Blue light is maximally absorbed at the upper surface and does not penetrate deeply into the leaf (Ramos & Lagorio, 2004). Red light penetrates deeper into the leaf inducing fluorescence over the entire cross section although a significant proportion of fluorescence is reabsorbed (Gitelson et al. 1999) resulting in an overall decrease in fluorescence intensity. Blue-induced fluorescence is emitted from the surface of the leaf only with very little re-absorption resulting in a higher fluorescence intensity and a strong, low noise fluorometer signal (Ramos & Lagorio, 2004).

The use of blue excitation light wouldn't be suitable for some experiments. Cyanobacteria contain specialised antennae known as phycobilisomes (PBSs) which are not thought to absorb blue light. Therefore, cyanobacteria have a reduced photosynthetic efficiency under blue light (Luimstra et al. 2018) and the use of a red excitation light would be applicable.

The emission characteristics of each LED relative to the absorption characteristics of chlorophyll *a* are shown in Figure 1. Figure 2 shows the fluorescence detection characteristics relative to the chlorophyll fluorescence emission spectrum for each variant of FMS-300.

FMS-300-B is fitted with a different fluorescence detector filter to the other variants, allowing fluorescence emission above 645 nm to be captured (Figure 2). This provides an extremely low-noise fluorescence signal since it transmits a significant proportion of the 685 nm emission peak. All other variants detect fluorescence above 680 nm (Figure 2).

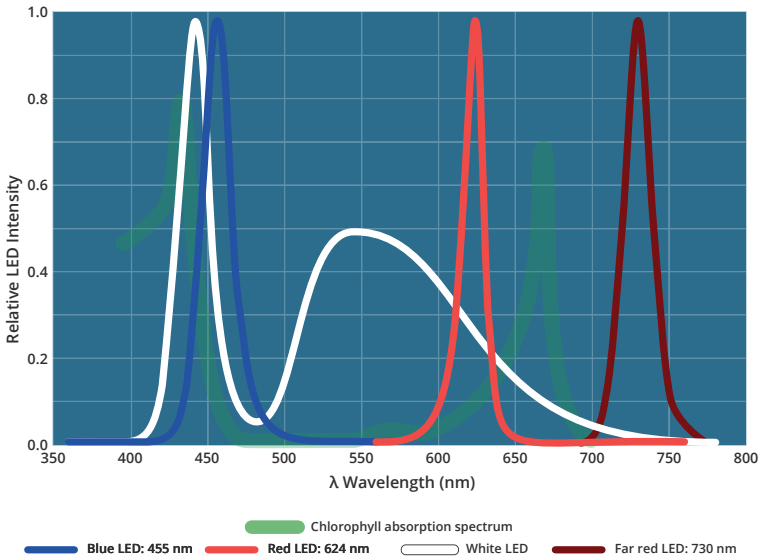


Figure 1: Emission characteristics of FMS-300 LEDs relative to the chlorophyll a absorbance spectrum.

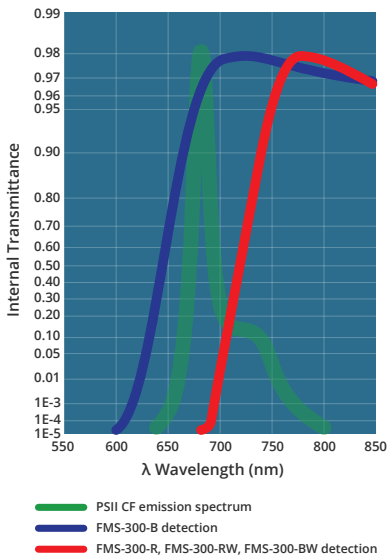


Figure 2: Detector characteristics of FMS-300 variants relative to the chlorophyll a emission spectrum.

Technical Specifications

All light intensity specifications refer to incident illumination at the sample surface.

Control Unit

Measuring LED:	Pulse width: 400 nanoseconds. Pulse frequency: Slow kinetics: 10 Hz. Fast kinetics: Semi-log frequencies from 10 Hz-100 kHz.
Pulse intensity:	Up to 0.1 µmol m^{-2} s^{-1} in FMS-300-B/BW. Up to 0.058 µmol m^{-2} s^{-1} in FMS-300-R/RW. Adjustable in 0.001 µmol m^{-2} s^{-1} increments. With light guide, FMS/LG2: >0.25 µmol m^{-2} s^{-1}. With light guide, FMS/LG3: >0.4 µmol m^{-2} s^{-1}.
Measuring LED colour:	FMS-300-B/BW: Blue 455 nm (FWHM 27 nm). FMS-300-R/RW: Red 624 nm (FWHM 18 nm).
Actinic intensity:	Up to 3,000 µmol m^{-2} s^{-1}. Adjustable in 1 µmol m^{-2} s^{-1} increments. With light guide, FMS/LG2: >8,000 µmol m^{-2} s^{-1}. With light guide, FMS/LG3: >12,000 µmol m^{-2} s^{-1}.
Actinic LED colour:	FMS-300-B: Blue 455 nm (FWHM 27 nm). FMS-300-R: Red 624 nm (FWHM 18 nm). FMS-300-BW/RW: Ultra-white.
Sat. pulse intensity:	>20,000 µmol m^{-2} s^{-1}. Adjustable in 1 µmol m^{-2} s^{-1} increments. With light guide, FMS/LG2: up to 60,000 µmol m^{-2} s^{-1}. With light guide, FMS/LG3: up to 90,000 µmol m^{-2} s^{-1}.
Saturating LED colour:	FMS-300-B: Blue 455 nm (FWHM 27 nm). FMS-300-R: Red 624 nm (FWHM 18 nm). FMS-300-BW/RW: Ultra-white.
Far-red LED:	730 nm (FWHM 20 nm). Intensity >20 µmol m^{-2} s^{-1}. With light guide, FMS/LG2: up to 60 µmol m^{-2} s^{-1}. With light guide, FMS/LG3: up to 90 µmol m^{-2} s^{-1}.
Detector:	PIN photodiode, rapid peak-pulse tracking.
Detection range:	FMS-300-B: >645 nm. FMS-300-R/BW/RW: >680 nm.
Electronics:	Dual processors: ARM 32-bit microcontroller running real-time operating system. PIC 8-bit microcontroller for dedicated measuring LED sampling.
Fibre-optic cable:	Length 1 m, statistically randomised fibre bundles, 7 mm optical diameter at sample end.
Connections:	Optical connection for fibre-optic cable, 12 V DC socket, USB-C socket, MiniDIN socket for PTL-100.
Enclosure:	Shielded aluminium enclosure.
Dimensions:	150 (l) x 150 (w) x 85 mm (d). Weight 770g.
Communications:	USB 2.0. Cable type A - C.
Operating conditions:	0°C - 40°C. Non-condensing humidity.
Power supply:	12 V DC mains power.
Power consumption:	5.6 - 7.2 W. Max. 50 W.
Display:	4 line x 20 character blue LCD display.

Leafclips & Accessories

FMS/DLC:	Nylon 3D printed, 7 mm sample aperture, sliding shutter blade, 60° angle between fibre-optic & sample.
PTL-100:	Nylon 3D printed, leaf temp. & PAR (400-700 nm) sensor. Electrical connection to FMS-300. Open faced, 60° angle between fibre-optic & sample.
Manfrotto arm:	Mini Variable Friction Arm. 0.44 kg, length: 12 - 24 cm, max. payload: 3 kg.
Manfrotto clamp:	Nano Clamp. 0.097 kg. clamp range 13 - 35 mm, max. payload: 4 kg.
Transport case:	464 (l) x 366 (w) x 176 (d) mm. Weight: ~3 kg.

Software requirements

Operating System:	Windows 10 or newer.

Supplied with

FMS-300 control unit.
Fibre optic cable.
PTL-100 PAR temperature leafclip.
FMS/DLC pack of 10 dark-adaptation leafclips.
Fibre optic cable support arm & clamp.
12 V DC Power supply.
USB A - C connection cable.
Hard transport case.

Options/Accessories

FMS/DLC: Additional pack of 10 dark-adaptation leafclips.
FMS/LG1: 100 x 8 mm hexagonal light guide.
FMS/LG2: 50 mm light guide tapering to 4 x 4 mm (x2 light intensity).
FMS/LG3: 50 mm light guide tapering to 2.5 x 2.5 mm (x3 light intensity).
FMS/LPC: Liquid sample chamber.
FMS/LPC-CU: 4.5 ml Sample cuvettes.
FMS/LPC-FL: Magnetic followers for 4.5 ml cuvettes.
FMS/MAG: Magnetic stirrer for liquid sample chambers.
FMS-300/RS: Solid oak base retort stand with laser engraving.

References

Baker NR. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol. (2008);59:89-113. https://doi.org/10.1146/annurev.arplant.59.032607.092759. PMID: 18444897.

Neil R. Baker, Eva Rosenqvist, Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities, Journal of Experimental Botany, Volume 55, Issue 403, August 2004, Pages 1607–1621, https://doi.org/10.1093/jxb/erh196

Ramos, M.E., Lagorio, M.G. True fluorescence spectra of leaves. Photochem Photobiol Sci 3, 1063–1066 (2004). https://doi.org/10.1039/b406525e

Gitelson, Anatoly A., Claus Buschmann, and Hartmut K. Lichtenthaler. "The chlorophyll fluorescence ratio F735/F700 as an accurate measure of the chlorophyll content in plants." Remote sensing of environment 69.3 (1999): 296-302.

Luimstra, Veerle M., et al. "Blue light reduces photosynthetic efficiency of cyanobacteria through an imbalance between photosystems I and II." Photosynthesis Research 138 (2018): 177-189.

Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. Analysis of the Chlorophyll a Fluorescence Transient. In: Papageorgiou, G.C. and Govindjee, Eds., Chlorophyll a Fluorescence: A Signature of Photosynthesis, Springer, New York, 321-362 (2004).

Kramer, D.M., Johnson, G., Kiirats, O. et al. New Fluorescence Parameters for the Determination of QA Redox State and Excitation Energy Fluxes. Photosynthesis Research 79, 209–218 (2004).

Hendrickson, L., Furbank, R.T. & Chow, W.S. A Simple Alternative Approach to Assessing the Fate of Absorbed Light Energy Using Chlorophyll Fluorescence. Photosynthesis Research 82, 73–81 (2004).

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

Hansatech Instruments