MULTI-COLOR-PAM-II

ST-Kinetics and Multi-Wavelength Chlorophyll Fluorometer

The new MULTI-COLOR-PAM-II is a compact instrument that houses two quite different techniques: the PAM technique and single turnover flash kinetics (STK).

MULTI-COLOR-PAM-II Highlights

Two instruments in one house: non-modulated flash analysis and multi-color PAM-modulated applications

A complete portfolio of fast measurements (time resolution down to 0.3 µs) to probe the PS II donor side and the presence of Car-triplets and Multi-Color (PAM) measurements to probe the PS II acceptor side, the electron transport chain and photosynthetic activity.

Mixing of both techniques: e.g. application of saturating 3 µs flashes, where the decay kinetics of the induced fluorescence signal can be monitored by PAM measuring light with a logarithmically declining measuring light frequency.

Below an overview of possible applications of both instruments:

PAM **Q_A-kinetics up to overall Photosynthesis activity**	**Single Turnover Flash Kinetics (STK)** **Oxygen evolving complex up to PQ pool**
Pulse-modulated fluorescence	Non-modulated fluorescence
Dark decay measurements	Pump-probe measurements
MT Pulses/continuous light	ST-flashes
Saturation pulse quenching analysis	Saturation flash quenching analysis

PAM Q_A-kinetics up to overall Photosynthesis activity	Single Turnover Flash Kinetics (STK) Oxygen evolving complex up to PQ pool
Regulatory fluorescence quenching (NPQ) and complementary PS II quantum yields	Short-lived fluorescence quenchers ($P680^+$ (DQ) and Car-triplets (TQ))
Dark-to-light induction curves	ST-kinetics (STK)
Two wavelength detection F_V(I)	Period-4 oscillations in F_V(II)
Fast kinetics (50 µs to seconds) and Slow kinetics (seconds to hours)	Flash train-induced fluorescence changes
Sigma(II) (O-I_1 rise)	Sigma(II) (FRRF emulation)
Q_A– re-oxidation kinetics	S-state decay
PS II quantum yield	S-state distribution
Light saturation curves (LC)	Flash saturation curves
O-I_1-I_2-P/OJIP transients	Wide range of ST-intensities
PS II heterogeneity	ST-widths and ST-dark intervals
Mixed use of both techniques: e.g., applying an STK during an O-I_1-I_2-P transient	

More choice is an important keyword for the new MULTI-COLOR-PAM-II: the new STK (single turnover kinetics) flashlamp and fast detector add reactions on the PS II donor side and Car triplets to the already broad portfolio. Fast switching between PAM and ST-Kinetics measurements gives synergy, offering a mixing of both techniques. The instrument is also flexible enough to emulate FRRF-type measurements or to produce Ramp-Method-type saturation pulses.

The instrument was designed for the measurement of suspensions, but people working with leaves were also not forgotten. The previous model had a leaf clip that can still be used for the new instrument. In addition, the STK-flashlamp also has a detector and can be used as a stand-alone application for leaf measurements.

Single Turnover Kinetics (STK) configuration for leaf measurements (see below)

Different users may have different needs. The MULTI-COLOR-PAM-II caters to this by offering different configurations.

Possible configurations:

- **The Multi-Color (PAM) configuration**: the multi-color-emitter (MCP-II-E) and its detector (MCP-II-D1 or MCP-II-D2ST)
- **Two Wavelength Detection (PAM) configuration (suspensions)**: a second detector (either MCP-II-D1 or MCP-II-D2ST) is added to allow the detection of fluorescence at e.g. < 710 nm (mainly PS II) and > 700 nm (PS II + PS I), simultaneously. A leaf clip allowing two wavelength detection will be available in the near future.
- **Full configuration**: STK-flashlamp (maximal flash intensities > 1.0 mol photons m^{-2} s^{-1}) and combi-detector (MCP-II-EDST + MCP-II-D2ST) allowing detection of signals down to approx. 100 ns are added to the Multi-Color (PAM) configuration. This gives access to PS II donor side reactions.
- **Single Turnover Kinetics (STK) configuration for suspension measurements:** STK-flashlamp (MCP-II-EDST) in combination with the combi-detector (MCP-II-D2ST).
- **Single Turnover Kinetics (STK) configuration for leaf measurements:** The STK-flashlamp as stand-alone application: the STK-flashlamp contains a detector for fluorescence measurements from the sample surface, which makes it ideal for the measurement of leaves.

In the Multi-Color (PAM) configuration, the user has access to information about the wavelength dependent effective PS II antenna size: Sigma(II), reactions on the PS II acceptor side (Q_A^- re-oxidation) and electron flow along the electron transport chain (O-I_1-I_2-P/OJIP transients) but also to Saturation Pulse quenching analysis, dark-light induction and recovery curves, as well as light response curves, and this all for 5 different excitation and measuring light wavelengths (440, 480, 540, 590, 625 nm) plus white light that can be applied in any combination. This has the advantage that someone working, e.g., with diatoms can excite these organisms in the green and in the case of cyanobacteria either choose 625 nm to excite the phycobilisomes or 440 nm to excite the chlorophylls of the core antenna.

Not only the different wavelengths for fluorescence excitation and detection make the Multi-Color (PAM) configuration stand out. Other important features are:

- The high sensitivity of the instrument, which allows measurements with optically thin samples, i.e., in the absence of intensity gradients of the various light qualities and largely avoiding wavelength-dependent fluorescence reabsorption – **light gradient free**.
- **Free choice of detection wavelengths** using optical filters, e.g., enabling detection of fluorescence >700 nm, enriched in PS I fluorescence F(I), and < 710 nm, enriched in PS II fluorescence F(II), that can be measured simultaneously under equal conditions.
- Characterization of the same state of a given sample by comparative PAM and flash kinetics (STK) measurements.
- The almost complete freedom to create trigger and script files means that the experiments that can be designed are mainly limited by the creativity of the user.

New features of the MULTI-COLOR-PAM-II

The new elements of the MULTI-COLOR-PAM-II add whole new domains to the measurement portfolio of the instrument, making it even more a multi-function fluorometer.

- **High time resolution:** The new STK-detectors provide a time resolution of 0.3 µs.
- **Time-resolved flash responses:** the MULTI-COLOR-PAM-II is the first commercially available instrument with which the fluorescence yield during a saturating µs flash can be measured and carotenoid triplet quenching (TQ) and donor-side dependent quenching (DQ) can be differentiated.
- **Highly precise:** For detailed analysis of the flash responses their timing is very precise and reproducible, and the form of the flashes approaches a rectangle, with the LEDs needing about 0.5 µs to reach full intensity. A special routine is provided for flash-profile correction.
- **Extremely intense flashes:** Flash intensities of more than 1000000 µmol 440 nm photons m^{-2} s^{-1} can be achieved with the EDST emitter-detector unit (yielding more than 1 excitation per µs).
- **Pump-probe:** Double flash experiments with variable dark intervals Δt (from 1 µs to 10 ms) allowing highly flexible relaxation measurements of various forms of quenching.

- **Period-4 oscillations:** Flash frequencies of up to 100 Hz (10 ms time interval) can be used for flash trains to probe the S-states of the oxygen evolving complex.
- **Leaf measurements:** Although the instrument was originally designed for suspension measurements, the EDST emitter-detector (STK-flashlamp) unit can also be used by itself to measure leaves. In addition, a configuration will be available soon in which two emitters are placed under a 45° angle relative to the multi-color emitter, which allows two wavelength detection of fluorescence emitted by leaves.
- **STK embedded in PAM recording:** Thanks to fast switching [8-10 µs switching time] between fast non-modulated and PAM measurements an ST can be placed anywhere along a traditional – e.g. O-I_1-I_2-P or Slow Kinetics recording, with the resulting STK revealing details on the state of PS II at the moment of the flash. All this taken together makes the MULTI-COLOR-PAM-II a very all-round fluorometer allowing the user to probe and monitor PS II and the photosynthetic electron transport chain in many different ways.

Configurations

Suspension and leaf configurations

Scheme of a complete configuration for suspension measurements (a) and stand-alone application of the STK flashlamp unit for leaf measurements (b) (see Klughammer, Schlosser and Schreiber (Photosynth Res 2024: https://doi.org/10.1007/s11120-024-01101-w) for a full description).

In the schematic version of the stand-alone application (see above), the leaf is placed in a gas flow chamber.

A leaf clip for the simultaneous measurement of two fluorescence wavelengths will be introduced soon. A prototype with the Multi-Color Emitter Head in the center and two Detectors placed under a 45° angle relative to the emitter, is shown below.

The two-wavelength fluorescence detection of a barley leaf with this configuration is illustrated below.

This leaf clip will replace the leaf clip configuration of the first-generation MULTI-COLOR-PAMs.

However, the leaf clip developed for the first-generation MULTI-COLOR-PAM can still be used in combination with the Multi-Color Emitter-Detector Units of the MULTI-COLOR-PAM-II.

Application

Example experiments from the three application domains

The software splits the applications of the MULTI-COLOR-PAM-II in 3 parts:

- Photosynthetic Activity related applications like induction (+ recovery) curves and light curves, but also manual measurements.
- Script-based experiments like O-I_{-1}-I_2-P/OJIP transients, re-oxidation kinetics of Q_A^- following a single turnover flash, Sigma(II) determinations.
- Flash-based experiments like period-4 oscillations, Car-triplet decay and induction kinetics, $P680^+$

The first two parts represent PAM-applications, and the last part represents Single Turnover Kinetics (STK) applications.

Example of a Light Curve/Quenching Analysis

The first figure gives an example of a light curve recording of the complementary PS II quantum yields, Y(II)+Y(NPQ)+Y(NO)=1. where the light intensity was first increased and then decreased again. Due to memory effects the kinetics induced by the decreasing light intensities may differ from those of the increasing light intensities. This phenomenon is called hysteresis. The here observed full reversibility of light-induced lowering of Y(II) and increase of Y(NPQ) is characteristic for physiologically healthy samples.

Examples of Fast Kinetics (PAM) measurements

Two wavelength measurements of O-I_{-1}-I_2-P transients

An example of a fast PAM-application is the simultaneous measurement of O-I_1-I_2-P transients in two different wavelength domains: < 710 nm and > 700 nm, where the fluorescence measured at wavelengths < 710 nm is mainly PS II fluorescence and the >700 nm fluorescence is a potential mix of PS II and PS I fluorescence. First a measurement of a dilute suspension of Chlorella cells (440 nm ML and MT) taken from Klughammer et al. (2024).

The O-I_1 rise (curves normalized to I_1) is the same for both wavelengths. A difference is observed between I_2 and P: the I_2-P rise is more pronounced at F>700 nm compared with F<710 nm.

Below, a barley leaf is measured using 440 nm measuring and actinic light using the two-wavelength configuration for leaves (see Configurations).

The two measurements were again normalized to I_1 (all Q_A reduced). Again, the I_2-P rise is more pronounced at F>700 nm compared with F<710 nm. The somewhat slower O-I_1 rise kinetics reflect the fact that self-absorption at F<710 nm is higher than at F>700 nm and, therefore, F>700 nm originates from relatively deeper layers in the leaf, where the effective actinic light intensity is lower.

Sigma(II) determination

The parameter Sigma(II) reflects the effective cross section of the PS II antenna. The Sigma(II) determination (and its wavelength dependence) is another fast PAM-application. There are three criteria on the basis of which one can judge if the O-I_1 fit used for the Sigma(II) determination was good: 1. The fit should describe the fluorescence rise well, 2. The obtained fit parameters should be physiologically relevant and 3. The obtained Sigma(II) values should be independent of the light intensity. Here, the Sigma(II) values were observed to increase with the age of the cultures used.

For this experiment, the connectivity parameter J was fixed to 1.2, the value obtained by Anne and Pierre Joliot in 1964. The dataset shows near perfect fits, reasonable parameter values and in essence an independence of the light intensity at high light intensities, yielding well-defined O-I_1 kinetics.

STK, single turnover flash kinetics, applications

Car-triplet decay

The next figure shows a set of measurements on the basis of which the Car-Triplet decay kinetics can be determined. The dataset illustrates the precision of the timing of the flashes and the ability of the flash lamp to give two equally strong flashes 1 µs apart.

Flash length and fluorescence induction

Another example is a double flash experiment in which the length of the first flash was varied and the second flash is given 40 µs after the first flash.

Flash trains and flash patterns can tell us something about the S-states, the redox states of the manganese cluster on the donor side. They can also tell us something about the effects of different intensities of far red.

Period-4 oscillations

Period-4 oscillations in either F_0, F_M or F_V level can be derived automatically by the software (figure taken from Klughammer et al. 2024).

In coffee leaves, FR1 illumination already leads to a strong damping of the period-4 oscillations in the variable fluorescence. In such cases, the MULTI-COLOR-PAM-II allows a further reduction of the effective FR-intensity to 10% of FR1, which for this coffee leaf strongly reduced the effect on the S-states.

Combining STK and PAM measurements

In the next example it is shown how a mixing of STKs and PAM measuring light allows the combination of the precise, intense and short STKs and PAM measuring light allowing the monitoring of fluorescence decay in darkness.

3 µs STK flashes applied to a dilute *Chlorella* sample inhibited by DCMU (blue) or uninhibited (red) embedded in a PAM measurement. The ML-frequency declined logarithmically from 100 kHz to 10 kHz starting 100 µs after the flash (figure taken from Klughammer et al. 2024).

Accessories for MULTI-COLOR-PAM-II - Suspension Configuration

Spherical Micro Quantum Sensor US-SQS/WB

Exact light measurements in suspensions (but also in air) can be carried out by the spherical micro quantum sensor US-SQS/WB. The sensor has a 3.7 mm diameter sphere as the entrance optics. When the sensor is connected to the Control Unit MCP-II-C , data will be acquired and processed by the PamWin-4 software.

Temperature Control Unit US-T

The US-T unit consists of a heat-transfer head with a cooling/heating Peltier element, and a separate power-and-control unit. The heat-transfer head is mounted on top of a Walz optical unit (ED-101US-type) so that the dip of the rod is in touch with the suspension investigated. The achievable temperature spread in suspensions is about 30 K; absolute temperatures depend on ambient temperature.

Download the manual for detailed information.

Miniature Magnetic Stirrer PHYTO-MS

Settling of particles is prevented by using a miniature magnetic stirrer (US-MS). The stirrer is mounted directly beneath the sample cuvette. A rotating magnetic field created by the stirrer tip moves a miniature magnetic stir bar in the cuvette. The stirrer is connected to the MULTI-COLOR-PAM-II Control Unit (MCP-II-C). Stirring can be switched on and off by the PamWin-4 software.

Temperature Control Block ED-101US/T

For measurements under defined temperatures, the temperature control block ED-101US/T can be mounted on the optical unit ED-101US/MD. The block consists of an inner flow-trough metal part which is slightly pressed on the sample cuvette by a spring mechanism, and an external foam part for temperature insulation. Temperature control is achieved by an external flow-through water bath (not included) connected to the temperature block.

Accessories for MULTI-COLOR-PAM-II - Leaf Configuration

Optical Unit for Leaf Measurements MCP-BK

This optical unit is designed for measurements of leaves or flat photosynthetic surfaces. The unit features a clip to position leaves optimally for fluorescence measurements. The clip has a port for a Mini Quantum Sensor US-MQS/WB.

Mini Quantum Sensor US-MQS/WB

A cosine-corrected mini quantum sensor measures light intensities which are relevant for plant leaves or flat surfaces. When the sensor is connected to the control unit MCP-C, data will be acquired and processed by the PamWin-3 software.

Leaf Support MCP-II-LS

For stand-alone measurements of leaves with the STK flashlamp (MCP-II-EDST), using a Linear Positioning System, the leaf has to be supported on the bottom side to keep it in place. The MCP-II-LS is a simple accessory that can play this role.

Specifications for MULTI-COLOR-PAM-II

Power-and-Control-Unit MCP-II-C
General design: ARM microcontroller (480 MHz), Fast Kinetics up to 128,000 points with 14 bit resolution, ST Kinetics up to 15,000 points with 14 bit resolution, unlimited storage for Slow Kinetics
Sockets: 4 sockets for measuring light and actinic light of MCP-II-E Multi-Color Emitter Head and measuring light/auxiliary detector and ST supply MCP-II-EDST, 2 sockets for signal detection by MCP-II-D1 and MCP-II-D2DST Detector Heads, charge socket or Battery Charger MINI-PAM/L, output socket for PHYTO-MS Miniature Magnetic Stirrer, 4 BNC sockets for 5 V trigger in and trigger out signals and ST trigger in and trigger out signals, input socket for US-SQS/WB Spherical Micro Quantum Sensor or US-MQS/WB Mini Quantum Sensor, input socket for auxiliary devices, USB socket
Communication: USB 2.0, USB 3 compatible
User interface: Windows computer with PamWin-4 software
Power supply: standard: no sealed lead-acid battery; Battery Charger MINI-PAM/L (100 to 240 V AC)
Dimensions: 31 cm x 16 cm x 33.5 cm (W x H x D)
Power consumption: 6 W
Weight: 3.64 kg
Operating temperature: -5 to +40 °C

Multi-Color Emitter Head MCP-II-E
Chip-on-board multi-wavelength measuring light LED emitter: 400, 440, 480, 540, 590, 625 nm for pulse-modulated measuring light; 20 intensity settings and 14 14 pulse frequency settings
Chip-on-board multi-wavelength actinic LED array: 440, 480, 540, 590, 625 and 420-640 nm (white) for continuous actinic illumination, max. 5000 µmol photons m^{-2} s^{-1}; saturating single turnover flashes, max. 200000 µmol photons m^{-2} s^{-1}; multiple turnover pulses, max. 12000 µmol m^{-2} s^{-1} PAR, adjustable between 1 and 800 ms
Far-Red LED: peak wavelength 730 nm
Dimensions: 10.5 cm x 5.5 cm x 7 cm (L x W x H)
Weight: 500 g (incl. cables, 1 m long)

Multi-Color Detector Head MCP-II-D1
Signal detection: PIN photodiode with special pulse preamplifier for measuring fluorescence changes with maximum time resolution of 10 µs
Filter box: for up to 14 mm filter thickness
Standard detector filter: long-pass filter >650 nm (3 mm RG 665) plus short-pass filter SP 710
Dimensions: 6.9 cm x 9.8 cm x 6.4 cm (L x W x H)
Weight: 355 g (incl. cables, 1 m long)

Emitter Head for fast Fluorescence Measurements MCP-II-EDST
Signal generation and detection: High quality 450 nm ST-flashes of variable duration and intensity up to 1 mol photons m^{-2} s^{-1}; Actinic light (450 nm); Far Red light (740 nm); STK-detector for surface detection of direct fluorescence with sub-µs time resolution
Dimensions: 15.4 cm x 5.2 cm x 7 cm
Weight: 570 g (incl. cables, 1.2 m long)

Combined Detector Head for Double Channel and Fast Fluorescence MCP-II-D2DST
STK & PAM-Detector (switchable): includes: PAM-detector equivalent to MCP-II-D1 and STK-detector for non-modulated fluorescence with sub-µs time resolution
Filter box: for up to 14 mm filter thickness
Standard detector filter: long-pass filter >650 nm (3 mm RG 665) plus short-pass filter SP 710
Dimensions: 6.9 cm x 9.8 cm x 6.4 cm (L x W x H)
Weight: 550 g (incl. cables, 1.2 m long)

Power Supply MINI-PAM/L
Input: 90 to 264 V AC, 47 to 63 Hz
Output: 19 V DC, 3.7 A

Operating temperature: 0 to 40 °C

Dimensions: 15 cm x 6 cm x 3 cm (L x W x H)

Weight: 300 g

It should be noted that the MULTI-COLOR-PAM-II does not contain a battery anymore.

Optical Unit for Suspensions ED-101US/MD

Design: Black-anodized aluminum body with central 10 x 10 mm standard glass cuvette; ports for attachment of the Emitter/Detector Heads MCP-II-E, MCP-II-D1, MCP-II-EDST and MCP-II-D2DST, and Miniature Magnetic Stirrer PHYTO-MS

Weight: 750 g

Stand ST-101

Stand for mounting the Optical Unit ED-101US/MD (suspensions) or the Linear Positioning System 3010-DUAL/B (leaves) or MCP-II-BK (leaves)

Transport Box PHYTO-T

Design: Aluminum box with custom foam packing for MULTI-COLOR-PAM-II and accessories

Dimensions: 60 cm x 40 cm x 34 cm (L x W x H)

Weight: 5 kg

System Control and Data Acquisition

Software: PamWin-4 System Control and Data Acquisition Program for operation of the instrument via PC or laptop, data acquisition and analysis

Saturation Pulse Analysis

Measured: Ft, F_0, F_M, F, F_0' (also calculated), F_M'. Time-resolved Saturation Pulse: Fast polyphasic rise and decay kinetics (time resolution up to 10 µs). PAR using Spherical Micro Quantum Sensor US-SQS/WB or Mini Quantum Sensor US-MQS/WB

Calculated: F_0' (also measured), F_V/F_M and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate), C/F_0 (constant fraction of F_0 not constituting PS II chlorophyll fluorescence)

Fitting Routines

Fitting routine for the fast fluorescence O-I_1 to determine the functional absorption cross-section of the PS II antennae (Sigma) needed for the determination of PS II-specific electron transport rates.

Fitting routine for exponential decay (e.g. Q_A^- reoxidation after a single turnover flash) or rise of a signal with up to three exponentials.

Choice of two fitting routines for light curves (determination of cardinal points α, I_k and ETR_{max}).

Computer Requirements

Operating system: Microsoft Windows 10 (32 and 64 bits) and 11; computers that can run these operating systems, can also work with PamWin-4.