

# DIVING-PAM-II

Underwater Fluorometer  
with Miniature Spectrometer



Measuring Aquatic Photosynthesis in its Natural Habitat

**WALZ**

# DIVING-PAM-II

## Underwater fluorometer with miniature spectrometer

The predecessor of the DIVING-PAM-II (the "DIVING-PAM") has been proven as reliable and robust chlorophyll fluorometer for studying photosynthesis in and under water. To date, more than 500 scientific papers reporting on measurements with the DIVING-PAM have been published.

The DIVING-PAM-II continues the manifold tested design of its predecessor, but it possesses significantly advanced data acquisition and instrument control by employing state-of-the-art optical and electronic components.

The DIVING-PAM-II fluorometer permits measurements of photosynthesis down to 50 m water depth. All functions of the instrument can be controlled by 10 infrared reflection switches.

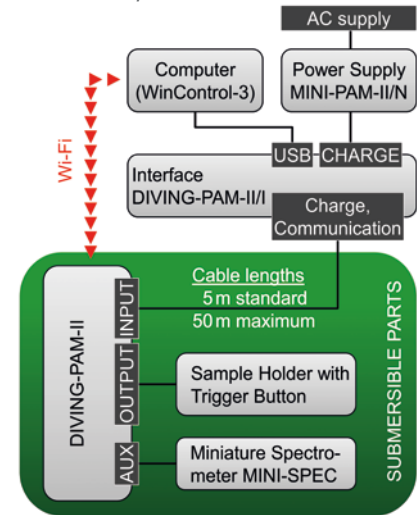
The innovative Miniature Spectrometer MINI-SPEC is part of the basic DIVING-PAM-II system. It adds a new level of information to studies of photosynthesis.

The DIVING-PAM-II also features an energy-saving B/W screen which displays instrument status and measured data. The transfective screen is readable even under sunlight. For long-term monitoring, the instrument can be operated by a computer via a special underwater cable. Wi-Fi access allows convenient download of data.

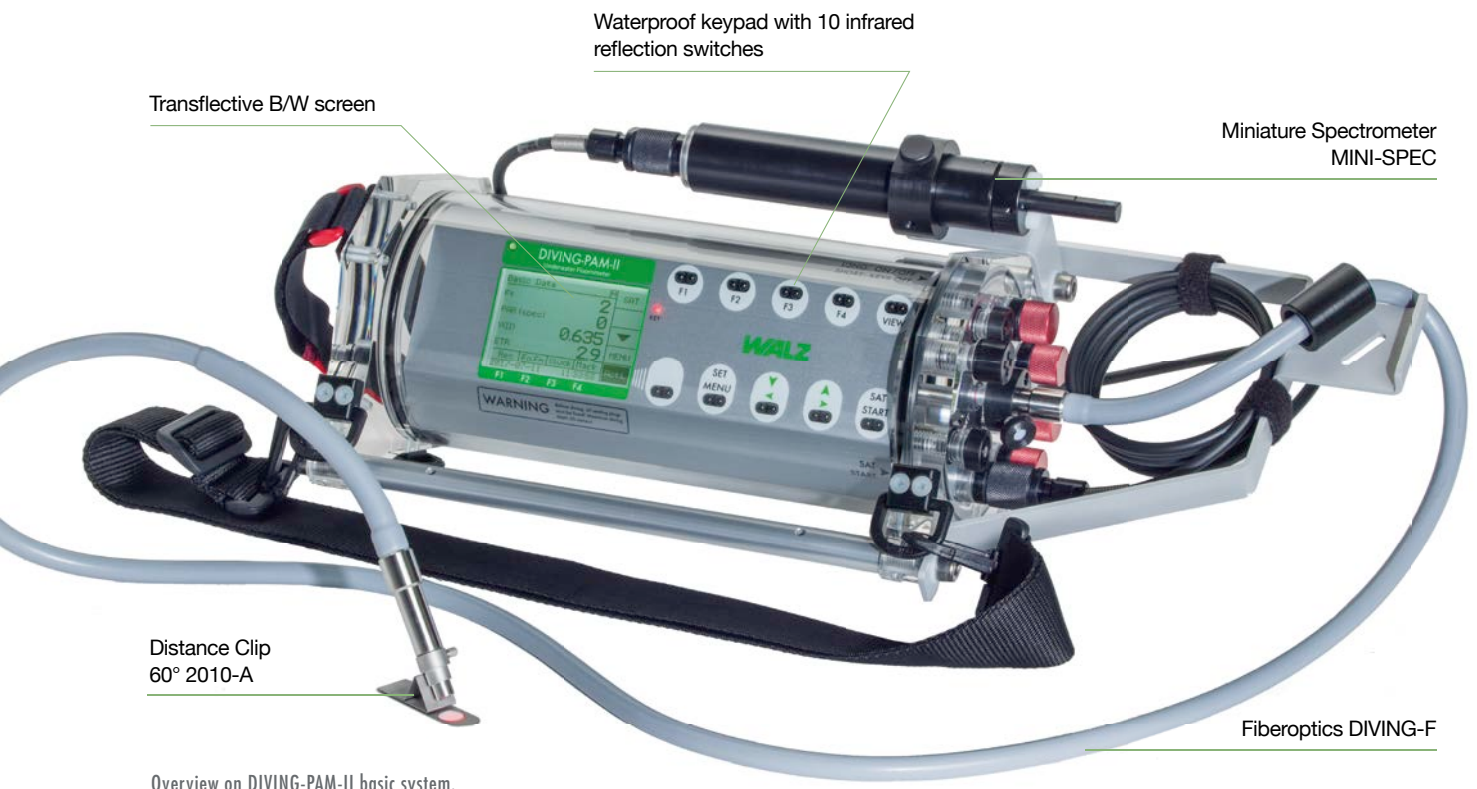
In dry environment, accessories of the MINI-PAM-II fluorometer can be operated in conjunction with the DIVING-PAM-II. Only a special adapter cable is needed. For example, oxygen measurements with suspensions can be performed in the lab using the Fiber-Optic Oxygen Meter FireStingO2.

Soon available will be a submersible optode-type oxygen sensor for marine and limnological research *in situ*.

DIVING-PAM-II System



Submersible parts are drawn inside the color box. Cable lengths for remote control of the DIVING-PAM-II are indicated.



Overview on DIVING-PAM-II basic system.

# Miniature Spectrometer MINI-SPEC

One device for spectra of PAR, reflectance and fluorescence emission

The Miniature Spectrometer MINI-SPEC is part of the basic system. It has been included in response to the interest of researchers to know the actual spectral environment in which plants and algae grow.

The heart of the MINI-SPEC forms a very compact detector unit consisting of a grating monochromator and a CMOS image sensor.

The configuration for measuring spectra of PAR can be easily converted to the setup for reflectance and fluorescence measurements.

The MINI-SPEC possesses 3 internal light sources: a white tungsten lamp for determining light reflectance in the visible range, as well as a blue and a green LED to excite fluorescence emission spectra.

To measure the PAR acting on the sample, the Universal Sample Holder DIVING-II-USH is equipped with an adjusting ring which positions the light-diffusing disk of the MINI-SPEC at sample level. Values of PAR are automatically calculated from spectral data.



Miniature Spectrometer MINI-SPEC and Sample Clip of Universal Sample Holder DIVING-II-USH.



During transport or when surfaces are inspected, the Miniature Spectrometer MINI-SPEC can be conveniently attached to a dedicated holder of the DIVING-PAM-II. The software provides the opportunity to record a light spectrum with each saturation pulse analysis.



Spectrometer module

Zenith Polymer® reflectance standard

Magnetic sample clip

Entrance Optics SPEC/P for spectra of PAR

The spectrometer module acts as a radiometer when connected to the entrance optics SPEC/P; the module measures reflectance and fluorescence emission spectra when the magnetic leaf clip is mounted.

# DIVING-PAM-II/B & DIVING-PAM-II/R

Two fluorometer versions using either blue or red measuring and actinic light

## DISTINCTIVE FEATURES

Microsecond timing enables the DIVING-PAM-II fluorometer to use the same high-power LED as source for PAM measuring light, actinic light and saturation pulses. Measuring light corresponds to  $\mu$ s flashes of constant amplitude, actinic light is quasi-constant light employed to drive photosynthesis, and saturation pulses temporarily saturate primary photosynthesis so that all photosystem II reaction centers are “closed”.

The color of light emitted by the high-power LED distinguishes the BLUE from the RED version of the fluorometer (Fig. 1). The BLUE version (DIVING-PAM-II/B) possesses a blue LED emitting maximally around 474 nm which is replaced by a red LED emitting maximally around 654 nm in the RED version (DIVING-PAM-II/R).

Both versions have a second LED providing far red light for specific excitation of photosystem I.

Another difference between the two versions is the spectral window for fluorescence detection. The BLUE version detects fluorescence at wavelengths  $> 630$  nm but the RED version detects fluorescence at wavelengths  $> 700$  nm (Fig. 2).

## BLUE OR RED VERSION?

Its extended range for fluorescence detection makes the BLUE version more sensitive than the RED version. In samples with high chlorophyll contents, a large part of the short wavelength fluorescence, which potentially can be detected by the BLUE version, is reabsorbed by chlorophyll. Hence, in such samples the sensitivity of the BLUE version is only slightly better than that of the RED one.

The DIVING-PAM-II can be used to investigate, e.g., cyanobacterial mats. Cyanobacteria often absorb poorly in the blue.

Therefore, in studies of cyanobacteria the RED version is normally preferred over the BLUE version which shows low signal to noise ratios with cyanobacteria.

The blue actinic light source of the DIVING-PAM-II/B excites the broad short wavelength band of the major light-harvesting complex of photosystem II in higher plants and green algae (LHC II). Red light of the DIVING-PAM-II/R excites the comparably minor long-wavelength band of the LHC II. Hence, if LHC II excitation is important, the BLUE version might be advantageous.

Blue is absorbed by blue light photo-receptors which can stimulate responses like chloroplast relocation in higher plants and sessile algae. Chloroplast relocation can affect the fluorescence signal by changing the efficiency of light absorption. This effect is difficult to distinguish from other fluorescence quenching mechanisms. Choosing the RED version excludes such blue light effects.

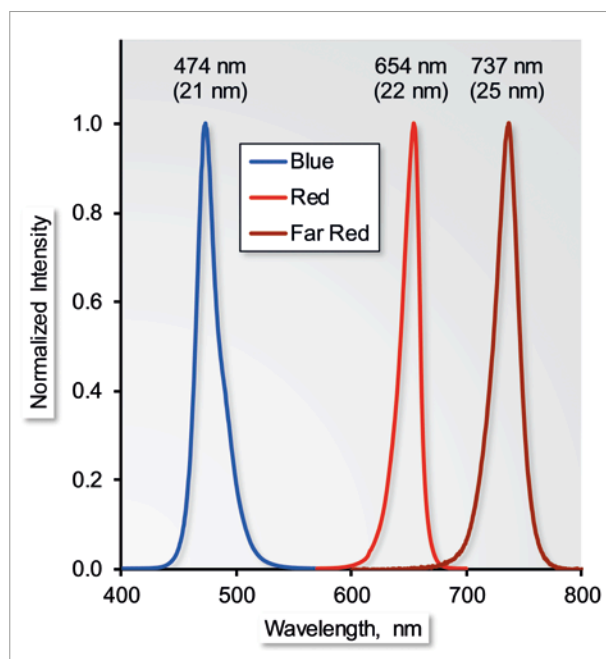


Figure 1: Typical LED emission spectra normalized to their maxima. The blue curve corresponds to the spectrum of the blue LED of the DIVING-PAM-II/B, the red curve represents the red LED in the DIVING-PAM-II/R. Both DIVING-PAM-II versions possess a far-red LED which emits maximally above 700 nm (rightmost curve). Peak wavelength and full width at half maximum (in brackets) are indicated.

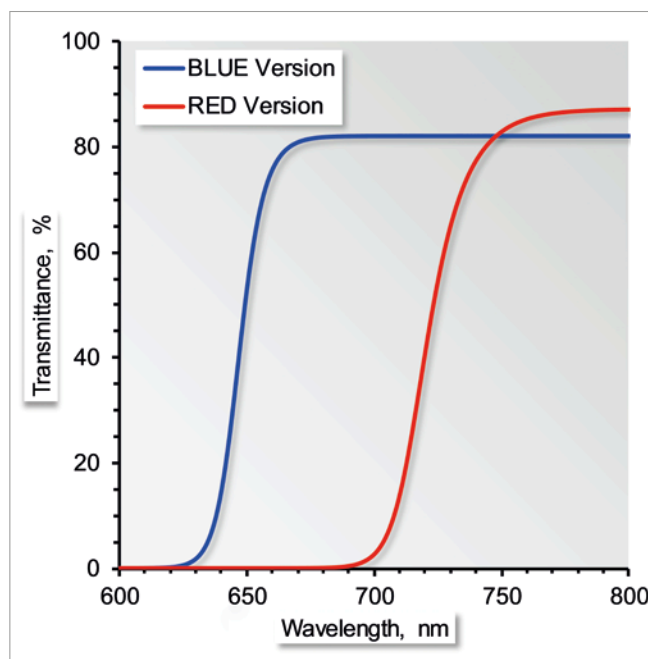


Figure 2: Transmittance spectra of detection filters in the DIVING-PAM-II/B (blue line) and DIVING-PAM-II/R (red line).

# Accessories

## Extending the capacity of the DIVING-PAM-II

### UNIVERSAL SAMPLE HOLDER DIVING-II-USH

The DIVING-II-USH sample holder is designed for the specific requirements of underwater investigations of samples like sea grass, macroalgae, and corals. The sample holder permits single-hand operation of the DIVING-PAM-II by triggering measurements via a release button integrated in the handhold. A special mount positions the Miniature Spectrometer MINI-SPEC parallel to the sample level.

Robust trigger and spectrometer cables as well as the fiberoptics are bundled by a nylon-mesh-cover so that harmful bending of the fiberoptics can be largely avoided. The DIVING-II-USH sample holder is designed to quantify relative electron transport rates (ETR) driven by natural light when PAR and Y(II) are measured in parallel. Using a special configuration which blanks out external light, the ETR driven by defined intensities of internal light can be studied.



### DIVING-LC

The leaf clip permits dark-acclimation of small areas of macro algae and leaves. The DIVING-PAM-II fiberoptics combined with the adapter DIVING-DA fits exactly on top of the DIVING-LC. With the fiber positioned, the sliding shutter of the DLC-8 can be opened so that  $F_0$  and  $F_M$  level fluorescence can be measured without interference by ambient light.



### SURFACE HOLDER DIVING-SH

The DIVING-SH accessory has a central port to accommodate the DIVING-PAM-II fiberoptics. For long-term measurements of bulky objects, the DIVING-SH can be attached to the sample by three rubber bands equipped with end hooks.

### MAGNET SAMPLE HOLDER DIVING-MLC

Clip for dark-acclimation of flat samples consisting of two magnetic parts. One part has a fiberoptics port which is closed by a flexible black rubber cap with a central slit. For measurements of dark-acclimated samples, the fiberoptics is pushed through the cap's slit so that natural light does not arrive at the sample and the dark-acclimated state is maintained.

### UNDERWATER CABLES DIVING-K25/-K50

For remote-control of the DIVING-PAM-II, reliably performing underwater cables either 25 m (DIVING-II/K25) or 50 m (DIVING-II/K50) long are available. The 50 m cable is supplied with the charger DIVING-II/L15 which delivers an increased voltage to efficiently charge the DIVING-PAM-II in the presence of the elevated resistance of the DIVING-II/K50.



# Example of Application

## Measuring simultaneously PAM fluorescence, PAR, leaf temperature, and relative humidity

### THE IDEA

Combining the DIVING-PAM-II fluorometer and the Miniature Spectrometer MINI-SPEC into one basic system is a new concept which was created to improve the understanding of how photosynthesis is influenced by both light intensity and light quality.

Because of positive feedback obtained for the MINI-SPEC for the DIVING-PAM-II, the spectrometer has been made compatible with the MINI-PAM-II fluorometer. In this case, the spectrometer is an accessory denoted MINI-SPEC/MP.

### REALIZATION

Objective of development was a submersible spectrometer with the capacity of measuring light spectra. Beyond this goal, it turned out that simple modifications convert the original setup into a spectrometer measuring reflectance and fluorescence.

Now, the Miniature Spectrometer MINI-SPEC can acquire spectral information on photon flux density, sample reflectance, and sample fluorescence emission.

### EXAMPLES

Three examples from the wide field of applications of the Miniature Spectrometer MINI-SPEC are given.

Figure 3 illustrates how light spectra change with water depth. Figure 4 shows how individual pigments contributing to the sample's reflectance properties can be identified. Figure 5 gives an example of how chlorophyll concentration affects the shape of fluorescence emission spectra.

### Examples

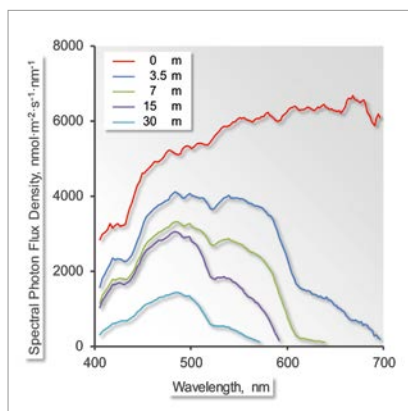


Figure 3: Absorption of light by water affects the spectral properties of radiation available for aquatic photosynthesizers. The spectra in Figure 3 were recorded above the water surface and at various depths down to 30 m. With increasing depth, intensities in the red spectral range decreased more than in the blue spectral range. Hence, photosynthesis has to acclimate to blue-enriched low light at greater depths. Measurements performed by Sabrina Walz and Jonathan Richir at La STARESO (Station de Recherche Océanographiques et sous-marines), Corsica, France.

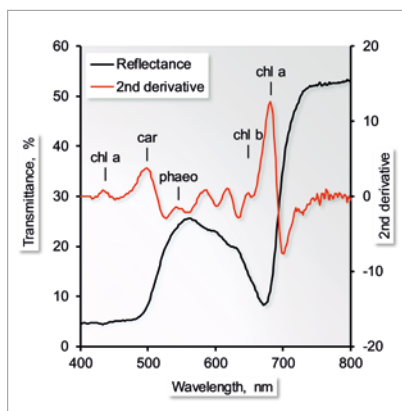


Figure 4: Reflectance spectra contain information on the pigments of the sample. Often, specific absorption peaks are distinguishable only in the derivative of the original spectrum. Here, a reflection spectrum of a senescing leaf was recorded, and its second derivative was calculated. The maxima indicated are chlorophyll *a* (chl *a*), carotenoids (*car*), phaeophytin (*phae*), and chlorophyll *b* (chl *b*).

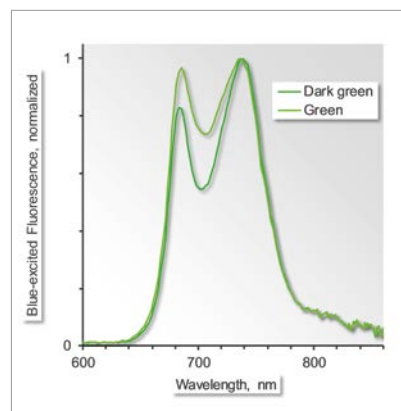


Figure 5: The shape of fluorescence emission spectra is affected by the chlorophyll content of a leaf (Buschmann C (2007) *Photosynth Res* 92, 261–271). Reabsorption of fluorescence at wavelength < 700 nm is the major factor determining the ratio of short to long-wavelength emission peaks. Figure 5 shows the fluorescence emission spectrum of a leaf with moderate chlorophyll content (Green) and of a leaf with high chlorophyll content (Dark green). Spectra are normalized to the long-wavelength maximum.

## TECHNICAL SPECIFICATIONS DIVING-PAM-II/B AND DIVING-PAM-II/R

### OPTOELECTRONIC UNIT DIVING-PAM-II/B (BLUE VERSION)

**Measuring light:** Blue LED (470 nm  $\pm$  8 nm tolerance), standard modulation frequencies 5 to 25 Hz adjustable in increments of 5 Hz and 100 Hz, measuring light PAR at standard settings = 0.05  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Fluorescence at wavelengths longer than 630 nm is measured

**Actinic light:** Same blue LED as for measuring light, maximum actinic PAR = 3000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , maximum PAR of saturation pulses = 6000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  adjustable at increments of 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$

### OPTOELECTRONIC UNIT DIVING-PAM-II/R (RED VERSION)

**Measuring light:** Red LED (655 nm  $\pm$  5 nm tolerance), modulation frequencies and PAR as described for DIVING-PAM-II/B. Fluorescence at wavelengths longer than 700 nm is measured

**Actinic light:** Same red LED as for measuring light, maximum PAR of actinic light and saturation pulses as described for DIVING-PAM-II/B

### FIBEROPTICS DIVING-F

**Design:** Randomized 70  $\mu\text{m}$  glass fibers forming a single plastic shielded bundle with stainless steel adapter ends

**Dimensions:** Active diameter 5.5 mm, outer diameter 8 mm, length 150 cm

**Weight:** 340 g

### POWER SUPPLY MINI-PAM-II/N

**Input:** 100 V to 240 V AC, 50 to 60 Hz

**Output:** 12 V DC, 5.5 A

**Operating temperature:** -5 to +45 °C (non-condensing)

**Weight:** 350 g including cables

### PC INTERFACE BOX DIVING-PAM-II/I

**Housing:** Aluminum case with USB-B port, socket for power supply MINI-PAM-II/N, and waterproof 6-pole socket for RS-485 communication

**Function:** Connects computer and DIVING-PAM-II. RS-485 serial data communication is used between box and DIVING-PAM-II, USB communication is employed between interface box and computer. Recommended maximum cable lengths: 50 m RS-485 cable, 2 m USB cable. Standard USB-A to USB-B cable included

**Dimensions:** 9.7 cm x 6.3 cm x 3.5 cm (L x W x H)

**Weight:** 270 g

**Operating temperature:** -5 to +40 °C

### OPTOELECTRONIC UNIT DIVING-PAM-II/B & DIVING-PAM-II/R

**Fluorescence detection:** PIN photodiode protected by long-pass and a short-pass filters, 12 bit signal resolution

**Pressure and temperature:** Piezo-resistive pressure sensor and temperature sensor. Pressure is converted in meters of diving depth, range 0 to -50 m, displayed at 0.1 m intervals. Temperature, range -10 to +60 °C, displayed at 0.1 °C intervals. Sensor module with gel protection and antimagnetic stainless steel cap

**Data storage:** Flash memory, 8 MB, providing memory for more than 27,000 saturation pulse analyses

**Display:** Backlit 160 x 104 dots (78 x 61 mm) transfective B/W screen

**Control elements:** 10 infrared reflection switches, pushbutton for saturation pulses, pushbutton to switch device on/off and to lock/unlock reflection switches

**Wi-Fi:** IEEE 802.11 b/g/n (2.4 GHz), Access Point Mode

**Design:** Plexiglas tube with Plexiglas end plates, one with waterproof fiberoptics port Mountings: 2 anodized aluminum rods (diameter 15 mm) mounted parallel to the fluorometer body. Anodized aluminum holder for fiber optics. Connections for carrying belt and miniature spectrometer MINI-SPEC

**Ports:** AUX1 and AUX2, 4-pole, for miniature spectrometer MINI-SPEC; for laboratory use via special adapter: Fiber-Optic Oxygen Meter FireStingO2 or Leaf Clip Holder 2035-B. OUT1 and OUT2, 6-pole, input for trigger signal from Universal Sample Holder DIVING-II-USH; for laboratory use via special adapter: External LED Light Source 2054-L. INPUT, 6-pole, for RS-485 communication and charging of internal battery

**Battery:** Lead acid battery 8.0 V / 3.5 Ah (28 Wh) providing power for more than 1300 yield measurements

**Maximum diving depth:** 50 m

**Operating temperature:** -5 to +45 °C

**Dimensions:** Diameter 19 cm, length 39 cm

**Weight:** 3.9 kg

### UNDERWATER CABLE DIVING-PAM-II/K5

**Length:** 5 m

**Weight:** 500 g

### MINIATURE SPECTROMETER MINI-SPEC

**Design:** POM tube, at one side, port for light detection, port for fluorescence excitation by blue (452 nm max) or green (525 nm max) LEDs, and port for white light from a tungsten lamp for reflection measurements; at the opposite side, 4-pole underwater socket

**Spectrometer module:** Hamamatsu micro-spectrometer, spectral range: 400 to 800 nm, spectral resolution: between 8 and 10 nm. Maximum PAR: 4000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for illumination having spectral characteristics similar to sunlight

**Dimensions:** 3.25 cm diameter, 17.5 cm length max

**Weight:** 135 g

### MINI-SPEC SMALL PARTS

#### Flat Entrance Optics SPEC/P:

**Design:** Hard-anodized aluminum rod with internal light guide of 10 mm diameter and 50 mm length, at one end with lateral light entrance through a 5 mm diameter diffusor and the opposite end inserted in a mounting plate (diameter 33 mm, height 5 mm)

#### Fluorescence and Reflection Optics SPEC/R:

**Design:** Spectrometer cap consisting of POM: maximum diameter 35 mm, height 13 mm, weight 16 g. With central 5 mm x 16 mm groove accommodating Perspex light guides for blue and green for fluorescence excitation, and for white light for reflection measurements. With 3 mm diameter central drilled hole as light channel to the detector window of the spectrometer. Including sample cap to fix sample (maximum diameter 40 mm, height 10 mm, weight 8 g). Spectrometer and sample caps padded with foam rubber, both parts have magnets build-in to attract each other and, thus, hold the sample

#### PAR Calibration Block 000160101439:

**Design:** POM block with drill hole for entrance optics of the Miniature Spectrometer. Oriented at an angle of 60° and 90° relative to the spectrometer port are drill holes for the Fiber Optics DIVING-F.

**Dimensions:** 4.15 cm x 2 cm x 5 cm (L x W x H)

**Weight:** 40 g

### DARK LEAF CLIP DIVING-LC

**Design:** Three clips made of white plastic with gasket contact areas and sliding shutter for light-tight closure.

**Dimensions:** Diameter 3.2 cm, length 8 cm

**Weight:** 6.5 g

# TECHNICAL SPECIFICATIONS DIVING-PAM-II/B AND DIVING-PAM-II/R

## SOFTWARE WINCONTROL-3

**Program:** WinControl-3 System Control and Data Acquisition Program (Windows 7, 8, 10) for operation of DIVING-PAM-II via PC, data acquisition and data analysis

**Saturation pulse analysis:** Measured:  $F(t)$ ,  $F_{0'}$ ,  $F_{M'}$ ,  $F_{0'}$ ,  $F_{M'}$  (also calculated),  $F_{M'}$ , PAR, water temperature and depth (derived from pressure). Calculated:  $F_{0'}$  (also measured),  $F_v/F_{M'}$  and  $Y(II)$  (maximum and effective photochemical yield of PS II, respectively),  $q_L$ ,  $q_{pr}$ ,  $q_{N'}$ , NPQ,  $Y(NPQ)$ ,  $Y(NO)$  and ETR (electron transport rate)

**Fitting routines:** Two routines for determination of the cardinal points  $\alpha$ ,  $I_k$  and  $ETR_{max}$  of light curves

**Programmed features:** Automatic determination of signal offset for all light intensities and all gain levels. Automatic calibration of internal PAR sensor against PAR from MINI-SPEC sensor

**Communication protocol:** USB and IEEE 802.11 b/g/n

**Computer requirements:** Processor, 1 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0

## DISTANCE CLIP 60° 2010-A

**Design:** Metal clip with fiber holder and 11 mm diameter sample hole: 5.5 cm x 1.4 cm (L x W)

**Fiber holder:** 1.2 cm length, mounted 0.7 cm above base, with lateral screw to fix fiber optics. Angle between fiber optics axis and sample plane: 60°. Two spacer rings to vary the distance between fiber end and leaf surface

## SURFACE HOLDER DIVING-SH

**Design:** Holder made of grey PVC, equipped with 3 rubber bands and hooks to be attached to creviced surface (e.g. of coral); nylon screws for distance adjustment

**Dimensions:** 6 cm x 6 cm x 2.5 cm (L x W x H)

**Weight:** 95 g

## TRANSPORT CASE DIVING-PAM-II/T

**Design:** Rugged, hard plastic outdoor case with wheels, pull-out handle and custom foam packing

**Dimensions:** 57 cm x 47 cm x 27 cm (L x W x H)

**Weight:** 7.7 kg

## ACCESSORIES

### UNIVERSAL SAMPLE HOLDER DIVING-II-USH

**Design:** Plexiglas bar (15 cm x 4.5 cm) with upward curved end possessing a port for positioning at 60° or 90° relative to the sample level the DIVING-PAM-II fiber optics. Mounted to the curved end is a 5.5 cm x 7.5 cm (W x H) sample clip consisting of a Plexiglas plate (lower part) and an aluminum frame open to the top (upper part). Featuring a 10 cm long plastic grip with button for triggering measurements via a 1.5 m trigger cable. Including a 1 m long tubular net with zipper to keep together trigger cable and fiber optics. With holder to for spectrometer MINI-SPEC

**Dimensions:** 25 cm x 4.5 cm x 21 cm (L x W x H)

**Weight:** 380 g

### MAGNET SAMPLE HOLDER DIVING-MLC

**Design:** Two halves with ring magnets, one with fiberoptics adapter and split rubber hood (dark adaptation), the other serving as buoyancy body

**Dimensions:** Diameter 37 mm, height 48 mm

**Weight:** 60 g; floating underwater

### UNDERWATER CABLE DIVING-II/K25

**Dimensions:** 25 m length, 6 mm diameter

**Weight:** 1.25 kg

### UNDERWATER CABLE DIVING-II/K50

**Design:** Dimensions: 50 m length, 6 mm diameter

**Weight:** 2.5 kg

### Including charger DIVING-II/L15:

Input: 100 V to 240 V AC, 50 to 60 Hz. Output: 15 V DC, 4.65 A. Operating temperature: -5 to +45 °C (non-condensing). Dimensions: 13 cm x 5.5 cm x 3 cm (L x W x H). Weight: 350 g including cables

### SURFACE HOLDER DIVING-SH

**Design:** Holder made of grey PVC, equipped with 3 rubber bands and hooks to be attached to creviced surface (e.g. of coral); nylon screws for distance adjustment

**Dimensions:** 6 cm x 6 cm x 2.5 cm (L x W x H)

**Weight:** 95 g

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# WinControl-3 Software

Acquiring, managing and evaluating kinetic data, saturation pulse analyses and spectra

The DIVING-PAM-II can be operated stand-alone or by the WinControl-3 software running on Windows computers. All functions of WinControl-3 are available when the DIVING-PAM-II is operated standalone, except continuous signal recording.

WinControl-3 also operates the first-generation DIVING-PAM, many other PAM fluorometers (e.g. JUNIOR-PAM and MONITORING-PAM) and the Universal Light Meter ULM-500.

In addition to performing saturation pulse analyses and recording of spectra, WinControl-3 acquires continuously fluorescence data and signals from optional peripherals like oxygen, temperature and PAR sensors.

## MEASURED DATA

**Saturation pulse analysis:** Photosystem II quantum yield, photochemical and non-photochemical energy dissipation, and electron transport rates are automatically calculated.

**Light curve analysis:** Cardinal parameters of light response curves are derived by two different models.

**Additional output:** PAR (continuously calculated from light spectra), water temperature and depth (derived from pressure).

## UNIVERSAL EXPORT FORMAT

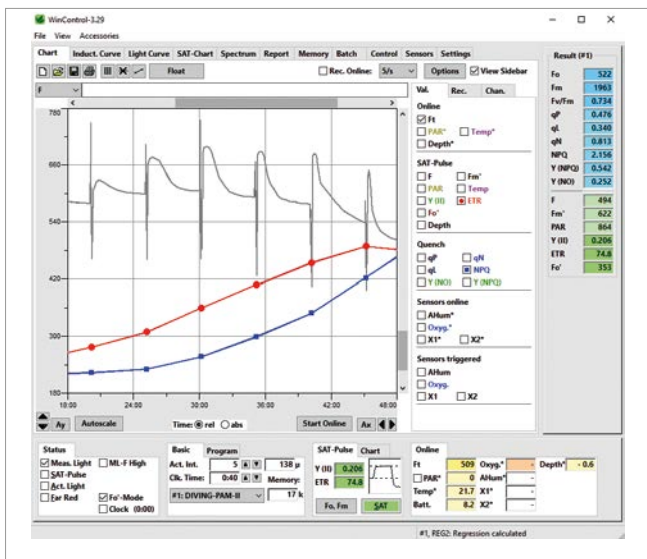
All measured data and cardinal parameters of light curves can be exported as csv files which can be read by spreadsheet software.

## EXPERIMENTAL ROUTINES

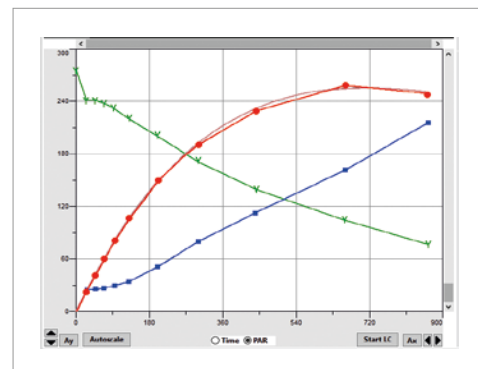
Automatic execution of light and induction curves. Automatic offset correction and calibration of internal light sensor. Repetitive triggering of fluorometer functions.

## PROGRAMMING EXPERIMENTS

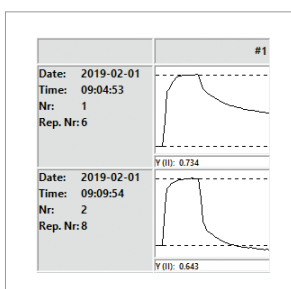
Execution of customized experiments using batch files. Easy generation of batch files by automatic transformation of manual operations into batch file commands.



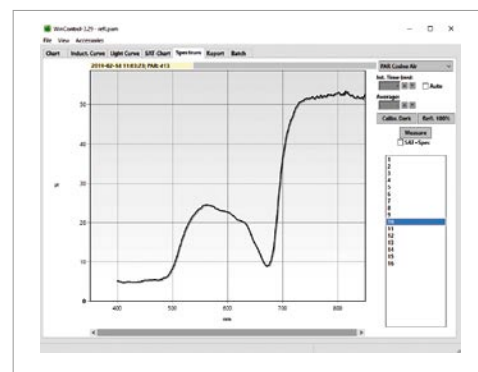
Window "Chart": Continuously recorded fluorescence (black line), ETR (red dots), and NPQ (blue dots) are displayed. Right panels: Control of data display and numerical results of saturation pulse analysis.



Window "Light Curve": A light curve experiment involving 10 light steps is shown. Blue symbols, NPQ. Green symbols, Y(II). Red symbols, ETR. The continuous graph describes the ETR as a function of PAR using the model by Platt et al. (1980) J Mar Res 38: 687-701.



Window "SAT-Chart": Fluorescence kinetics elicited by 2 consecutive saturation pulses.



Window "Spectrum": The reflectance spectrum of a light-green leaf was measured. In the range from 400 to 850 nm (Y axis range used: 0 to 60%).



## High Quality Instrumentation for Plant Sciences

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