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.

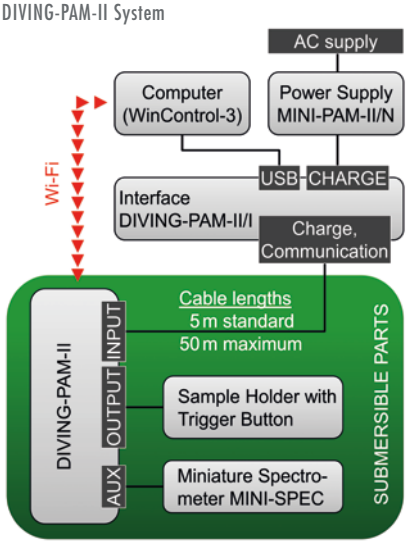
The innovative Miniature Spectrometer MINI-SPEC is part of the basic DIVING-PAM-II system. It adds a new level of information to studies of photosynthesis.

The DIVING-PAM-II also features an energy-saving B/W screen which displays instrument status and measured data. The transflective 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.

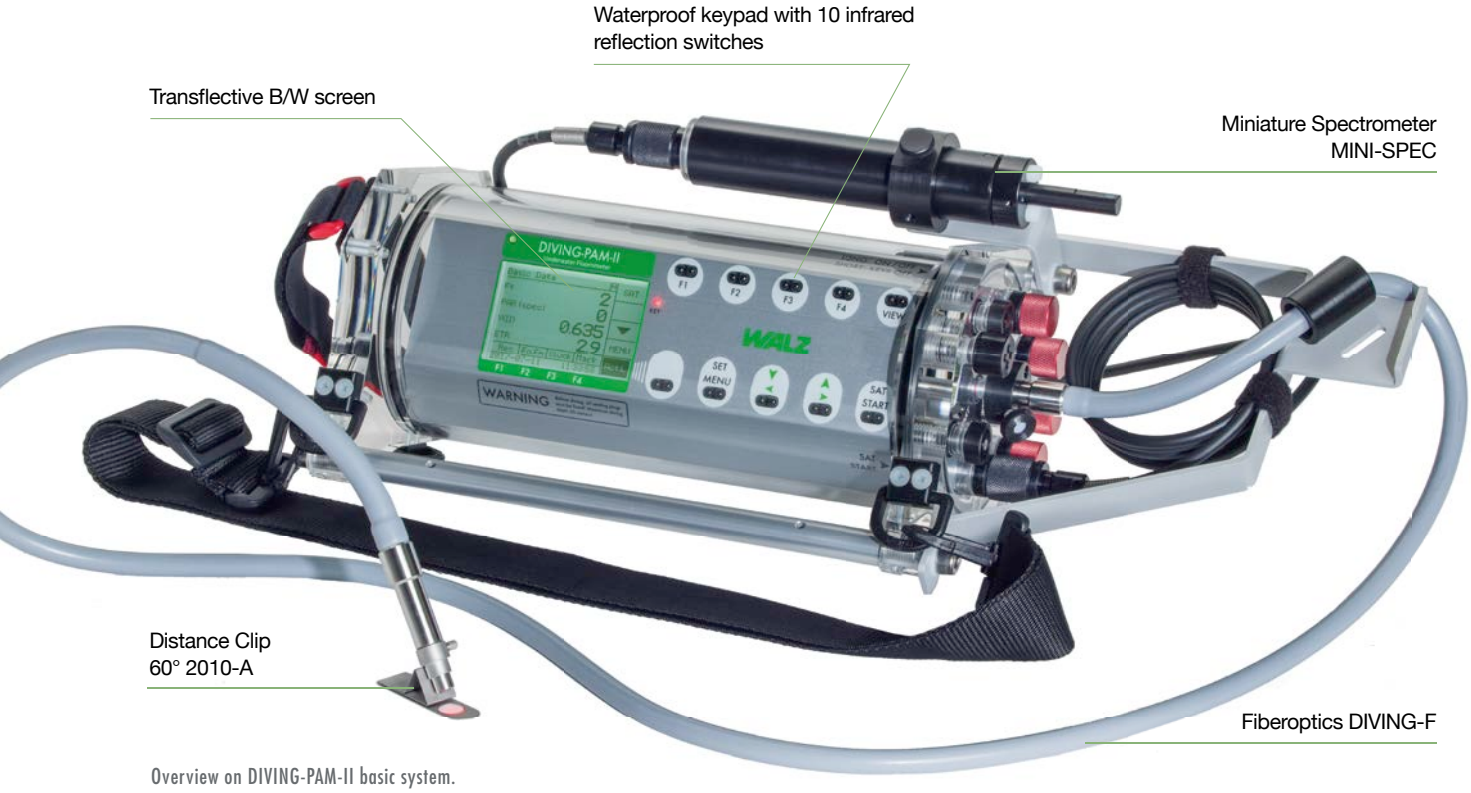
In dry environment, accessories of the MINI-PAM-II fluorometer can be operated in conjunction with the DIVING-PAM-II. Only a special adapter cable is needed. For example, oxygen measurements with suspensions can be performed in the lab using the Fiber-Optic Oxygen Meter FireStingO2.

Soon available will be a submergible 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 be 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.

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 µs 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 photoreceptors 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.

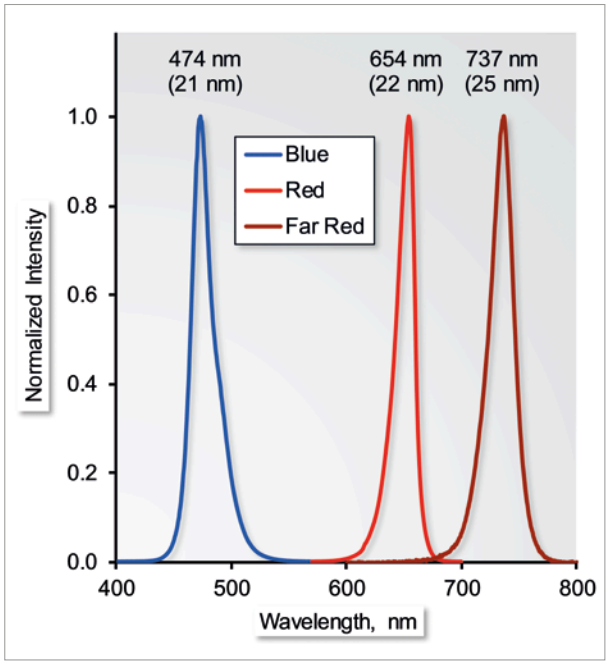


Figure 1: Typical LED emission spectra normalized to their maxima. The blue curve corresponds to the spectrum of the blue LED of the DIVING-PAM-II/B, the red curve represents the red LED in the DIVING-PAM-II/R. Both DIVING-PAM-II versions possess a far-red LED which emits maximally above 700 nm (rightmost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

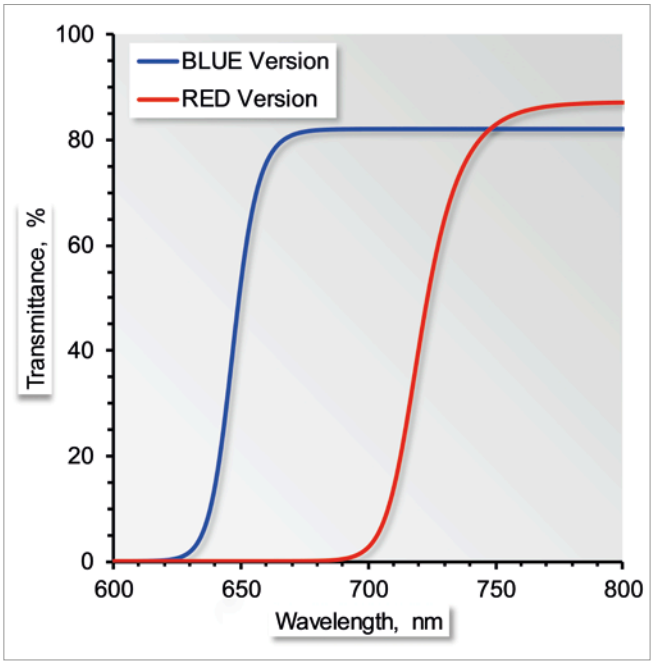


Figure 2: Transmittance spectra of detection filters in the DIVING-PAM-II/B (blue line) and DIVING-PAM-II/R (red line).

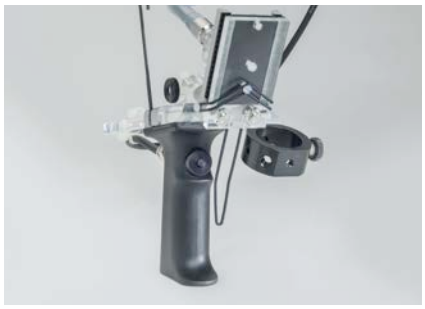
Accessories

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 measure 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 submergible 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

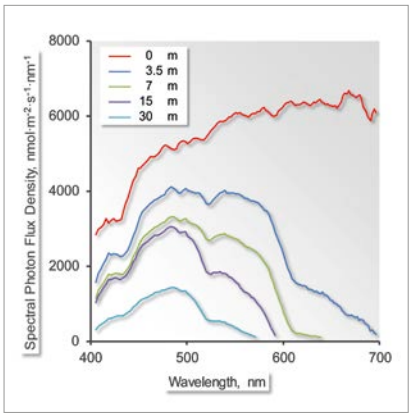


Figure 3: Absorption of light by water affects the spectral properties of radiation available for aquatic photosynthesizers. The spectra in Figure 3 were recorded above the water surface and at various depths down to 30 m. With increasing depth, intensities in the red spectral range decreased more than 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.

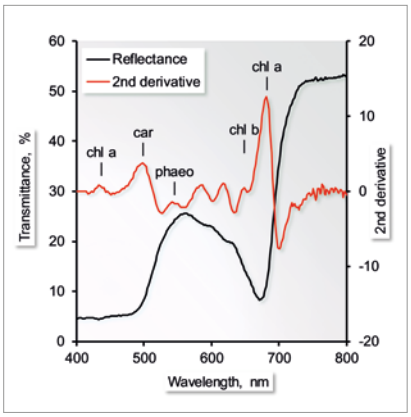


Figure 4: Reflectance spectra contain information on the pigments of the sample. Often, specific absorption peaks are distinguishable only in the derivative of the original spectrum. Here, a reflection spectrum of a senescing leaf was recorded, and its second derivative was calculated. The maxima indicated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

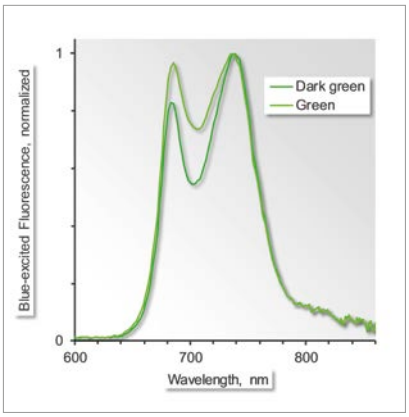


Figure 5: The shape of fluorescence emission spectra is affected by the chlorophyll content of a leaf (Buschmann C (2007) Photosynth Res 92, 261–271). Reabsorption of fluorescence at wavelength < 700 nm is the major factor determining the ratio of short to long-wavelength emission peaks. Figure 5 shows 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.