Points Relating to Fiberoptics Version of the DUAL-PAM-100

Heinz Walz GmbH • Eichenring 6 • 91090 Effeltrich • Germany
Phone +49-(0)9133/7765-0 • Telefax +49-(0)9133/5395
info@walz.com • www.walz.com

Points Relating to Fiberoptics Version of the Dual-PAM-100

Content

I General

The fiberoptics version of the Dual-PAM-100 is functionally fully equivalent to the standard "transmission version". The two versions employ the same hardware and software components. Major differences in the fiberoptics version with respect to the standard version are:

a) All optical components are integrated in the Power-and-Control-Unit, thus rendering the instrument more compact and portable.

b) As the Chlorophyll Fluorescence and P700 Emitter and Detector Units are fixed within the Control Unit, no alternative Emitter Detector Units (e.g. for measuring P515/535) can be connected.

c) P700 is measured in the "remission mode", i.e. the pulse-modulated Measuring Light is picked up by the fiberoptics, after multiple scattering and partial absorption within the sample, from the same side where it has entered.

d) The fiberoptics version is optimized for small sample areas; maximal signal quality is already obtained with 7 mm ∅ areas.

II Fiberoptics and distance to sample

The fiberoptics consist of three mixed fiber bundles, connecting to the built-in Optical Unit. The joint cross-section with an active diameter of 6.5 mm splits into

1) 4.0 mm (active ∅) carrying Red Actinic Light (Chip-On-Board LED Array)

2) 3.5 mm (active ∅) carrying Fluorescence and P700 Measuring Light, Far-Red light and Blue Actinic Light

3) 3.0 mm (active ∅) carrying the emitted fluorescence and remitted P700 Measuring Light to the photodiode detector.

The effective light intensities and signal amplitudes strongly depend on the distance between the fiberoptics and the sample. For standard applications a distance of 1 mm is recommended. With

increasing distance the intensities of Measuring and Actinic light decrease and the amount of fluorescence as well as of remitted P700 Measuring Light picked up by the fiberoptics declines. Both aspects contribute to a decrease of Fluorescence and P700 signals. While the Fluorescence signal normally is sufficiently high that even 10 mm distance give close to optimal results, the quality of the P700 signal decreases substantially between 1 and 10 mm distance. Nevertheless, under favorable conditions (in particular high chlorophyll content) satisfactory P700 measurements can be carried out even at 15 mm distance. With transparent samples, the signal amplitudes can be increased by placing the sample on a reflecting surface, so that the transmitted near-infrared P700 Measuring Light and chlorophyll fluorescence are reflected back via the sample into the fiberoptics.

The actinic intensities of the fiberoptics version were determined at the factory for standard 1 mm distance between fiberoptics and sample. The corresponding PAR lists are incorporated in the User Settings file Walz_Fiberoptics.DEF delivered with the instrument.

III User settings and standard optical geometry

While the same user software applies for fiberoptics and standard versions, due to the differences in effective light intensities and sensitivity, there are some differences in the default User Settings. Together with the instrument a CD with the user software is delivered, which contains the file Walz_Fiberoptics.DEF. This file has to be copied into the User Settings directory of the DualPAM folder, which already contains the standard User Settings file Walz.DEF optimized for the transmission version of the Dual-PAM-100. For users of the fiberoptic version, it is recommended to rename Walz_Fiberoptics.DEF by the standard name Walz.DEF, in order to avoid inadvertent mix-up. Besides the possibility of a reset of all instrument settings by opening the Walz.DEF or any other previously defined User Settings file, the Dual-PAM software also features "Set to default values" buttons for individual Settings windows (e.g. Actinic Light). It should be noted that the "default values" always correspond to the values in the file named Walz.DEF.

When Walz_Fiberoptics.DEF (or Walz.DEF after renaming) is opened (under Settings/Open User Settings), particular instrument settings are installed, which have proven optimal for standard measurements with the fiberoptics version, when the distance between fiberoptics and sample is 1 mm. An appropriate "distance tube" is provided, which can be mounted to the metal endpiece of the fiberoptics. Using this "distance tube" the standard 1 mm distance is assured, when the fiberoptics are positioned directly on top of a sample. For leaf samples a Dark-Clip is provided, which assures standard 1 mm distance between fiber optics and leaf. Major differences in default settings for fiberoptics vs. transmission versions are:

a) Fluo Gain 1 (Low) vs. 5 (High)

b) P700 Meas. Light Intensity 15 vs. 5

Furthermore, the PAR lists of Act. Red Light, Far Red Light, Act. Blue Light and Fluo Meas. Light are different.

IV Fiberoptics stand and different optical geometries

As pointed out above, the distance between the fiberoptics and the sample has a large effect on signal amplitudes. Therefore, in order to avoid signal fluctuations, it is essential that the sample and fiberoptics are rigidly fixed with respect to each other. For this purpose a Stand is provided, on

which the fiberoptics can be mounted and the metal endpiece fixed at defined distance to the sample. Besides the Dark-Clip, for leaf-like samples also a special magnetic holder is available, which is optimized for use with small *Arabidopsis* leaves.

The Fiberoptics Stand is also recommended for use in conjunction with the KS-2500 suspension cuvette (optional). Please note that for P700 measurements with suspensions relatively high chlorophyll content is required (at least 10 µg Chl/ml).

In principle, the fiberoptics version of the Dual-PAM-100 is also compatible with the Leaf-Clip-Holder 2030-B, which has been successfully used in the past in conjunction with the PAM-2000/2100 and MINI-PAM Portable Chlorophyll Fluorometers. The standard 60° version of this holder features a minimal distance of 7 mm between fiberoptics and leaf. While, as pointed out above, at this distance the P700 signal is suboptimal, it is still good enough to reliably assess the essential parameters of energy conversion in PS I in samples under natural daylight conditions. It should be noted, that in this geometry all intensities of internally generated Actinic Light applied via the fiberoptics are decreased by a factor of 3.5 with respect to the default PAR list for standard 1 mm distance. Hence, if use of the Leaf-Clip-Holder 2030-B and of internally generated Actinic Light is made, the PAR lists displayed under Settings and saved in the User Settings file Walz.DEF, have to be correspondingly modified and saved under a new name.