Robust O₂ Optode

(FSO2-II & FSO2-AK) Manual

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1 Safety Instructions

1.1 General Safety Instructions

The MINI-PAM-II and the Oxygen Meter FireSting-O2 (denoted hereinafter as "devices") and sensors should be used only by qualified personal.

Read safety and operating instructions in the manual prior to operation of the devices and sensors.

Follow safety and operating instructions of the manual, as well as the appropriate laws and guidelines for safety in the laboratory.

Keep devices and sensors outside the reach of children.

Keep devices away from water or high moisture areas.

Ensure that neither liquids nor foreign bodies get inside devices.

Keep devices and sensors away from dust, sand and dirt.

Do not put the devices and sensors near sources of heat.

Ensure sufficient ventilation.

Connect the devices only to the power source indicated in the operating instructions or on the device. If the devices are not in use, remove the mains plug from the socket.

The devices should only be repaired by qualified personnel. There are no serviceable parts inside the device. Please note that opening the housing will void the warranty!

The devices and sensors are not intended for medical or military purposes or any other safety-critical applications.

The devices and sensors must not be used for applications in humans; not for in vivo examination on humans, not for humandiagnostic or any therapeutic purposes.

1.2 Special Safety Instructions MINI-PAM-II

The MINI-PAM-II is a highly sensitive instrument which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual to avoid potential harm to the user and damage to the instrument.

The MINI-PAM-II can emit very strong light. To avoid harm to your eyes, never look directly into the light port of the MINI-PAM-II or its fiber optics.

1.3 Special Safety Instructions OXCAL

The OXCAL capsules for O_2 calibration contain sodium sulphite (Na₂SO₃). Before use, read instructions in the safety data sheet delivered with the OXCAL capsules.

OXCAL first aid measures

<u>General notes:</u> Take off contaminated clothing.



Following inhalation: Provide fresh air. In all

cases of doubt, or when symptoms persist, seek medical advice.

Following skin contact: Rinse skin with water/shower

<u>Following eye contact:</u> Rinse cautiously with water for several minutes. In all cases of doubt, or when symptoms persist, seek medical advice.

Following ingestion: Rinse mouth. Call a doctor if you feel unwell.

2 Extent of Delivery

2.1 Oxygen Package FSO2-II

Table 1: Extent of Delivery

	Item	Order Code		
1	FireStingO2 (includes items 1.1 to 1.4)	FSO2-II		
1.1	Oxygen Meter FireSting O2, Micro USB cable, 2 m long	120301666		
1.2	Optical Oxygen Robust Probe OXROB10-HS	120301665		
1.3	Adapter to mount the oxygen sensor on a Suspen- sion Cuvette KS-2500	246401014		
1.4	O-ring 3.00 x 1.50 UV	150702031		
2	Interface Converter for FireStingO2 (includes items 2.1 to 2.4)	FSO2-AK		
2.1	Proprietary connector cable (FSO2-AK Interface to MINI-PAM-II)			
2.2	One Phillips and one slotted screwdriver			
2.3	Perspex plate with holder for interface connector			
2.4	Three rubber (nitrile butadiene rubber, 10 x 2) and 3 silicone open O-rings (11 x 2) plus tool to position and remove O-ring			
3	USB flash drive, containing this manual, WinControl- 3 software, and Pyro Workbench software.			
4	OXCAL O2 Calibration Capsules (10 pieces)			

2.2 Accessories Required

Accessories Required Table 2: Item Order Code 1 Suspension Cuvette KS-2500 (includes items 1.1 to KS-2500 1.5) 1.1 246400514 **Fiberoptics Adapter** 1.2 3 Stir Bars 170201109 1.3 Retrieval Tool for Magnet Bars 246400914 1.4 Syringe Adapter 246400714 1.5 Metal Sleeve 246401114 2 Magnetic Stirrer with Fiberoptics Holder MKS-2500 MKS-2500 including Perspex base plate with stand bar to mount fiberoptics

3 Background

PAM fluorescence measurements in combination with saturation pulse analysis is well-established to analyze state and turnover of photosystem II [1]. Earlier than PAM fluorescence, oxygen measurements have been made commercially available to evaluate photosynthetic electron transport rates where these measurements have employed Clark electrodes [2]. Combining PAM fluorescence with oxygen measurements has been proven to provide a deeper understanding on photosynthetic performance under non-stressing and stressing conditions [3], [4], [5].

[1] Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynthesis Research 10: 51 – 62. https://doi.org/10.1007/BF00024185

[2] Delieu T, Walker DA (1972) An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. New Phytologist 71: 201-225. <u>https://doi.org/10.1111/j.1469-8137.1972.tb04068.x</u>

[3] Matthias Gilbert, Christian Wilhelm, Michael Richter (2000) Bio-optical modelling of oxygen evolution using in vivo fluorescence: Comparison of measured and calculated photosynthesis/irradiance (P-I) curves in four representative phytoplankton species. Journal of Plant Physiology 157: 307-314. https://doi.org/10.1016/S0176-1617(00)80052-8

[4] Duarte P, Ramos M, Calado G, Jesus B (2013) *Laminaria hyperborea* photosynthesis–irradiance relationship measured by oxygen production and pulse-amplitude-modulated chlorophyll fluorometry. Aquatic Biology 19: 29-44. https://doi.org/10.3354/ab00515

 [5] Drath M, Kloft N, Batschauer A, Marin K, Novak J, Forchhammer K
 (2008) Ammonia triggers photodamage of photosystem II in the cyanobacterium Synechocystis sp. Strain PCC 6803. Plant Physiology 147: 206–215. https://doi.org/10.1104/pp.108.117218 In the year 2015, Walz has introduced simultaneous PAM fluorescence and oxygen measurements with the MINI-PAM-II device. For oxygen measurements, an optical sensor ("optode", also called "optrode") was favored over the Clark electrode. The optode technology is superior to the Clark electrode because of its long-term stability of calibration, small dimension, oxygenfree operation, and fast response time (< 3 s, 90% signal change).

The oxygen-responsive element of the optode is a luminescent dye whose light emission is quenched by oxygen. In practice, however, not the luminescence intensity is measured but the phase shift of luminescence relative to the sine-modulated excitation light.

This manual introduces the "Optical Oxygen Robust Probe" which replaces the needle-type optode originally employed. Compared to the needle-type sensor, the new probe is less delicate, shows an improved signal to noise ratio, and a doubled lifetime.

The oxygen probe is part of the "FSO2-II" package which includes an oxygen meter and the interface "FSO2-AK" which makes the oxygen signal available to the WinControl-3 software.

The result of a typical light response curve experiment performed with the MINI-PAM-II + optode system is shown in Fig. 2.



Fig. 1: Principle of O₂ Detection

A, high luminescence intensity at low oxygen concentrations. B, low luminescence intensity at high oxygen concentrations. According to <u>www.pyroscience.com</u>, modified.



Fig. 2: Oxygen and Fluorescence Light Curve

4 System Setup



Fig. 3: System Overview

See Chapter 2 (page 5) for details. The water bath is not part of delivery.

4.1 Oxygen Logger Software

Do not connect the PyroScience logger to your PC before the Pyro Workbench software has been installed.

Install PyroScience logger software "Pyro Workbench & Data Inspector". The software is available on the Walz flash drive and can be downloaded from the PyroScience website:

https://www.pyroscience.com/en/downloads

Restart PC.

Connect the PyroScience logger to the computer using the Micro-USB cable provided. See Fig. 4 for ports. The USB communication does not work when the FSO2-AK interface is connected to the PyroScience logger. Connecting the oxygen probe is not required at this point. Launch Pyro Workbench & Data Inspector. Open the Settings Wizard by clicking on the device picture. Enter sensor code. (The settings will be entered later). The sensor code is written on the label of the sensor cable. Entering the sensor code adjusts the oxygen meter settings to the O_2 sensor and installs the factory sensor calibration.

Disconnect PyroScience logger.



Fig. 4: FireSting-O2

4.2 Temperature

The optode signal is temperature sensitive. With a thermometer connected to the FireSting-O2 logger, temperature effects could be automatically compensated. The optode used here has no temperature sensor and "Fixed Temperature" is the method of temperature compensation. Temperature must be entered

manually, and oxygen measurements must be carried out at constant temperature.

Constant temperature is achieved by connecting a circulating water bath to the KS-2500 cuvette. The KS-2500 cuvette has nozzles for tubes of about 8 mm inner diameter (Fig. 5). The water bath must have an exactly working temperature control. If the temperature regulation is adjusted at longer time intervals, the actual temperature may periodically fluctuate around the target temperature. Such fluctuations result in sawtooth-shaped oxygen signal (Fig. 6).

Safety note: Read and follow the safety instructions provided by the manufacturer of the water bath.



Fig. 5: KS-2500 Overview

The figure shows the KS-2500 cuvette with connections for the fiberoptics, the adapter (syringe or optode), and the water bath.



Fig. 6: Signal Interference

Periodic signal oscillation was caused by temperature pulses from a thermostatically controlled water bath. Data were recorded with a needle-type O_2 sensor

4.3 Cuvette Cleaning

Turning over the KS-2500 cuvette for sample change and cleaning is difficult. Cuvettes can be conveniently emptied using a commercially available aspiration system for laboratories. Take care that the tube end is narrow enough to prevent the stir bar from entering the aspiration tube. For example, a plastic pipette tip can be attached to narrow the tube end.

Alternative to an aspiration system, a gas washing bottle can be connected to the intake of an air diaphragm pump. Care must be taken that liquid does not spill into the pump, and it has to be made sure that the pump tolerates accidentally aspired liquids.

It is advisable to place the KS-2500 cuvette, as well as the aspiration system and a squeeze bottle (for cuvette rinsing) in a trough.

4.4 O₂ Probe Cleaning and Storage

Normal operation: Flush with water and let dry.

Remove deposits with detergent solution or 70% ethanol. Expose the sensor to the ethanol solution only for a short time. Flush with distilled water. Let dry.

Store at room temperature in darkness. Storage time > 3 years.



Fig. 7: Perspex Plate

Perspex plate with clamp for interface plug.

4.5 Connecting the Interface

Fasten the FireSting-O2 oxygen meter on the Perspex plate using the 4 screws enclosed (cf. Fig. 7).

Completely disassemble the clamp for the interface plug (Fig. 8, top). Check the correct orientation of the interface plug

relative to the interface port on the FireSting-O2 oxygen meter. Insert interface plug. Assemble clamp (Fig. 8). To link interface with MINI-PAM-II, connect the female end of the AUX cable with the interface and the male end with the leaf clip port or any of the two AUX ports (Fig. 9).

Connect Optode to fiber optics port. Avoid strong bending of the fiberoptics.



Fig. 8: Mounting the Interface Plug

Top left: Correctly positioned plug and parts for mounting (frame and locking piece with screws). Bottom left: Frame placed below plug, locking piece with one screw positioned. Bottom right: Optode Port.



Fig. 9: FSO2-AK Interface and MINI-PAM-II

4.6 The KS-2500 Cuvette

The KS-2500 side port can be fitted to use a microliter syringe or an optode (Fig. 10 and Fig. 11). In the first case, a metal sleeve at the bottom of the side port forms a narrow duct for the syringe needle.

To stabilize the needle, the syringe adapter is longer than the optode adapter. Both adapters can be locked by turning clockwise and unlocked by turning counterclockwise (Fig. 11). Locking the optode adapter also locks the optode.

4.7 Mounting the Optode

Insert optode in the optode adapter (cf. Fig. 11).

Slide the small O-ring a short distance over the optode.

Insert optode with adapter in the side port of the KS-2500 cuvette.

Turn the adapter clockwise until slight resistance is felt. (The optode should still be movable within the adapter.)

Position the optode so that the entire front disc is inside the sample chamber of the KS-2500 cuvette (see Fig. 11, bottom right). Visually examine the position of the sensor tip.

Lock optode (cf. Fig. 11, bottom left).



Fig. 10: Adapter

Left, optode adapter. Right, syringe adapter. (Not true to scale.)



Fig. 11: KS-2500 Cuvette

Top left and right, setup for use of syringe or optode, respectively. Bottom left, sense of rotation to lock/unlock the adapter for the syringe or the optode- Bottom left, positioning of the optode tip in the suspension. (Not true to scale.)

4.8 Completing the O2/Fluorescence Unit

Place the KS-2500 cuvette on the MKS-2500 Magnetic Stirrer (Fig. 13). Fill in 500 μ L of water. Put stir bar inside cuvette and visually examine stir bar rotation. Optimize rotational speed.

Fiberoptics Adapter

Place split O-ring in cuvette as schematically shown in Fig. 12. The split permits equilibration with atmospheric pressure and allows any existing bubbles to escape. O-rings made of rubber (nitrile butadiene rubber) or silicone rubber are provided. Both types are suited for work with suspensions of photosynthetic particles. When working with potentially reactive compounds, the highly inert silicone rubber rings may be advantageous.

Use 500 μL of suspension volume so that the liquid level is above the split O-ring.

Close cuvette by gently pressing the fiberoptics adapter on the O-ring. Visually control that the optical window of the adapter is fully immersed. In the presence of bubbles, lift the adapter just above the liquid surface and immerse again. Lock fiberoptics adapter by the set screw of the KS-2500 cuvette (Fig. 5, page 13).



Fig. 12: Suspension Level



Fig. 13: KS-2500 and MKS-2500

The stand for the fiberoptics is supplied with the stirrer MKS-2500.

5 Calibration

5.1 Preparation

<u>Factory Calibration</u> is active after having entered the sensor code (Section 4.1). Using factory calibration, test experiments can be performed. However, maximum accuracy requires two-point calibration.

Prior to calibration, all devices and sensors must be kept for at least 30 minutes under constant environmental conditions. The two-point calibration requires calibration liquids representing the upper and lower limit of the measuring range. When the sensor is placed into a new calibration standard, wait until the sensor reading is stable, then calibrate.

5.2 Zero % Calibration Liquids

Microalga suspension

A simple and safe way to reach 0% O2 is keeping a microalga suspension in the dark. All available oxygen will be consumed by respiration.

OXCAL

Part of delivery are 0% calibration capsules called OXCAL (Py-roScience, Aachen, Germany). One capsule gives 50 mL 0% calibration standard.

Use a 50 mL wide-neck container. Add magnetic stir bar and capsule. Fill up with demineralized water. DO NOT use saline water (e.g., seawater). Close container. Avoid air bubbles.

Place wide-neck container on a magnetic stirrer and stir until the salt is completely dissolved. Stop stirring and leave to stand for about 15 minutes. Immerse sensor into 0% calibration solution.

Let equilibrate and perform calibration. Do not store the sensor in this solution and rinse carefully after the calibration with demineralized water.

OTHERS

<> Water thoroughly bubbled with nitrogen gas (pass gas through an air stone).

<> Procedure according to:

Delieu T, Walker DA (1972) An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. New Phytologist 71: 201-225

"A few small crystals of sodium dithionite are added to stirred water in the cells which are then closed with the plungers. This reacts with dissolved oxygen according to the equation

 $Na_2S_2O_4 + O_2 + H_2O \rightarrow NaHSO_4 + NaHSO_3$

and since the reaction goes rapidly to virtual completion this procedure is equivalent, but more convenient, than prolonged flushing with O_2 -free nitrogen."

5.3 Hundred % Calibration Liquids

Water bubbled with air (pass air through an air stone connected to an air pump, e. g., an aquarium pump).

If an air pump is not available, fill water into a flask leaving about 50% air in the head space and shake it strongly for about 3 minutes.

5.4 User Interface

<u>Touchscreen.</u> The currently measured oxygen concentration is displayed on window "Primary Data" (Fig. 14). To calibrate, go to window "Oxygen Sensor Settings" (Main Menu \rightarrow Sensors \rightarrow Oxygen Sensor \rightarrow Oxygen Sensor Settings). Open Menu Settings and enter sensor configuration (see below). Then calibrate.

Primary Da	ta		% A	
Ft	583			SAT
Fo, F	1289		1	
Fm, Fm'	1590			
Fvm,YII	0.184			
ETR	32.5			
PAR	420	02	98.0	
Temp	26.4	X1	-	
Depth	0.1	X2	-	MENU
Rec	Fo,Fm	Clock	Mark	
2023-02-09	9	06:4	6:29	ACT.L.

Fig. 14: Primary Data

Oxygen Sensor Settings		
Meas. Interval (s)	2	
Blank Out LED	off	
Settings	\rightarrow	
Output Format	\rightarrow	\bullet
Calibrate 0%	\rightarrow	SET
Calibrate 100%	\rightarrow	3E1
		EXIT
dPhi (deg)		

Fig. 15: Oxygen Sensor Settings

WinControl-3

Go to window "Sensors" (Fig. 16), and click Start Calibration. In the field "O2 Calibration (Fig. 17), enter settings (see below). Then calibrate.

e view Accessories				
hant Induct. Curve Light Curv 2: MIN-PAM-II and USB - Ser Ext. PAR Sensor Active Without int. PAR Spectrometer Oxygen Sensor Sensor is attached Start Calibration Signal low Background too high Low Reference High Reference Ext. Act. Light Settings LED-off Mode Meas. Interval 1 K K	e SAT-Chart Spectrum Report h Mr: ccc with Comment Leafclip Leafclip is attached ADC Error Thermocouple Error	femory Batch Control	Sensors Settings	Result (#2) Fro Frv/Frm QP QL QN Y (NPO) F Frm' PAT PAT Y (II) ETR Fr' Fr'
itatus Meas. Light ML-F High SAT-Pulse Act. Light Stirrer Far Red C-Mode	Basic Program Act. Int. 8 ★★ 420 μ Cik. 0:30 ★★ Memory: #2: MINI-PAM-II ✓ 0.001 k	SAT-Pulse Chart Y (II) ETR	Online 123 Oxyg.* 243.4 ✓ PAR* Ø AHum* - Temp* X1* - Batt. 7.2 X2* -	

Fig. 16: Sensors Window

5.5 Settings

The following settings apply.

Temperature

Choose "Specify" and enter the temperature of current calibration liquid.

Pressure

Choose "Pyrosc. Int." to use the pressure sensor of the FireSting-O2 meter.



Humidity (calibration)

Choose "Specify" and enter 100%.

<u>Salinity</u>

Enter the dissolved salt content of the medium used for oxygen measurements (in g/L). Salinity plays a role only when using the output format μ mol/L (see the Pyro Workbench & Data Inspector manual by PyroScience). To measure oxygen in media containing high sugar contents, do not choose μ mol/L as format as the FireSting-O2 does currently not deal with the "salting-out

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effect" of sugars. A paper dealing with sugar-dependent solubility of O_2 is:

Rischbieter E, Schumpe A, Wunder V (1996) Gas Solubilities in Aqueous Solutions of Organic Substances. Journal of Chemical & Engineering Data 41: 809-812. https://doi.org/10.1021/je960039c

Units of calibration

Choose one of the three options provided for the water phase:

- Dissolved O₂ concentration, µmol/L.
- Partial pressure pO₂, mbar (=hPa).
- Percent air saturation, %.

Zero calibration

Immerse sensor in Zero % calibration liquid, wait for constant oxygen signal and press Calibrate 0%.

100 % calibration

After careful rinsing, immerse in 100 % calibration liquid, wait for constant oxygen signal and press

Calibrate 100 %.

Stability

The calibration of the sensor is rather stable. Still, it is a good practice to check the calibration at regular intervals.

Expected phase shift

As a rule of thumb, anoxic conditions will give about dphi=53, whereby ambient air will give about dphi=20. Dphi is the phase shift of light emission by the sensor relative to excitation light.

After calibration, rinse thoroughly with distilled water.

6 Hints

6.1 Signal Constancy

Add to cuvette 500 μ L of water containing a low level of dissolved oxygen. Close cuvette. Significant rates of diffusion of oxygen from ambient air into the cuvette appear as baseline drift. If this is the case, check setup. (Proper placement of Oring and fiberoptics adapter? Proper volume?)

6.2 Signal Amplitude

Optimize chloroplast or cell concentration to achieve changes in oxygen between 10 - 30% of full scale. If possible, work at intermediate oxygen concentrations. Ensure sufficient CO_2 supply.

6.3 Temperature Effects

Inefficient thermostatic control could result in slight warming of the sample by illumination with high actinic light intensities: this can affect the oxygen signal and would appear as actinic lightdependent baseline drift. To test for thermal constancy, measure cuvette temperature with a thermocouple (e.g. Mini Quantum/Temperature-Sensor 2065-M, Walz). Also, temperature effects can be tested using a suspension of heat inactivated chloroplasts (cf. Venediktov and Krivoshejeva 1984).

Venediktov PS, Krivoshejeva AA (1984) Effects of pH and deuterium oxide on the heat-inactivation temperature of chloroplasts. Planta 160: 200-203. https://doi.org/10.1007/BF00402854

6.4 Sensor Lifetime

The minimum number of measurements by a single oxygen sensor is 2,000,000 corresponding to almost 600 hours of measuring time for 1 Hz measuring frequency.

Switching off the oxygen sensor (by turning off the MINI-PAM-II) during experimental pauses extents the sensor lifetime.

6.5 Gas Bubbles

Short intervals of high rotational speed can release gas bubbles from the cuvette wall which then can escape through the O-ring gap (see next section).

6.6 Initial Oxygen Concentration

To measure photosynthesis with algae, establish reduced initial oxygen contents by mixing sample and degassed medium in the cuvette. Degassing can be achieved by, e.g., vigorous stirring under vacuum. The same applies to chloroplast suspensions except when paraquat (methyl viologen) is used as electron acceptor which results in net oxygen uptake during photosynthesis.

6.7 Chlorophyll Concentration

A frequently used chlorophyll concentration in oxygen measurements is 100 μ g/ml. Starting with this concentration, optimize your experiment to obtain clear changes in oxygen but, at the same time, keep the total change in oxygen moderate to exclude possible effects of varying oxygen concentrations on photosynthetic electron transport.

6.8 Evaluation

The primary optode signal is the oxygen concentration. The first derivative (dO_2/dt) provides information on photosynthetic rates.

At the time of writing this manual, the dO₂/dt is achieved by exporting oxygen data (right click on graph in Chart window) and calculating the continuous difference ($O_2(t+\Delta t)-O_2(t)$) of the oxygen signal in a spreadsheet program. Calculating the first derivative of the oxygen concentration will be implemented in the next software version.

The size of the Δt chosen affects the noise level and the signal response time. For example, a large Δt will smooth the signal but also dampen fast changes in oxygen rates. When selecting a Δt , bear in mind that the optode response time is < 3s. Longer values of Δt might obscure fast signal changes.

7 Specifications

7.1 F2O2-II FireStingO2 (1-Channel)

7.1.1 Oxymeter

Design: Aluminum case with 1 optical sensor port, 1 Pt 100 port, and 1 USB port. Integrated sensors: relative humidity and atmospheric pressure

Dimensions: 7.8 cm x 12.0 cm x 2.4 cm

Weight: 290 g

7.1.2 Optode

Measuring range: 0% - 500% air saturation (a. s.)

Accuracy: ± 0.1% a.s. at 5% a.s.

Resolution: 0.05% a.s. at 5% a.s.

Response Time: < 3 s for 90% signal change

Minimum Lifetime: 2.106 data points

7.2 F2O2-AK Interface Converter for FireStingO2

7.2.1 Interface Box

Design: Aluminum case with USB connector and fixed cable with MINI-PAM-II AUX connector

Dimensions: 5.5 cm x 3.0 cm x 2.0 cm

Weight: 55 g

7.2.2 Support

Design: Perspex plate with holder for interface connector

Dimensions (max.): 12.0 cm x 11.0 cm x 3.0 cm

Weight: 75 g

7.3 KS-2500 Suspension Cuvette and MKS-2500 Magnetic Stirrer with Fiberoptics Holder

See MINI-PAM-II manual

8 Guarantee

8.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

8.2 Conditions

This Guarantee shall not apply to:

- Any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
- Damage caused from improper packaging during shipment or any acts of God.

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- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps (halogen, LED), thermocouples, and underwater cables.
- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.
- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

8.3 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at <u>https://www.walz.com/support/repair_service.html</u> must be completed and returned to Heinz Walz GmbH, Germany.
- The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.

- All products being returned for guarantee service must be carefully packed and sent freight prepaid.
- Heinz Walz GmbH, Germany is not responsible or liable for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

8.4 Applicable Law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

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