pH*Optica*™ *micro*

Fiber Optic pH System
for pH microsensors

www.wpiinc.com

INSTRUCTION MANUAL

PC-controlled one-channel fiber optic pH system for pH microsensors; excitation wavelength of 470nm; quartz-quartz glass-fibers of 140 µm outer diameter connected by ST-fiber connectors;

World Precision Instruments

Table of Contents

1 Preface

Congratulations!

You have chosen a new innovative technology to read out chemo-optical sensors!

The pHOptica micro is a compact, portable, PC-controlled fiber-optic phase detection device to read out pH sensors. The data evaluation is PC supported.
The pHOptica micro was specially developed for very small fiber-optic microsensors. It is based on a novel technology, which creates very stable, internal referenced measured values. The pHOptica micro is useful to read out sensors with average luminescence decay times in the range of 1 µs to 0.6 ms.

Optical sensors (also called optodes) based on decay time measurements have important advantages over intensity based optodes:

- They are small
- They are independent on fluctuations of the light source
- Their signal does not depend on the flow rate of the sample
- They are less sensitive to photo bleaching and dye leaching
- They are not influenced by intensity fluctuations due to fiber bending

Therefore, they are ideally suited for the examination of small sample volumes, long term measurements in difficult samples, and for biotechnological applications.

A set of different microsensors, flow through cells and integrated sensor systems is available to make sure you have the sensor which is ideally suited to your application.

Please feel free to contact our service team to find the best solution for your application.

2 Safety Guidelines

PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS INSTRUMENT!

This device has left our works after careful testing of all functions and safety requirements.

The perfect functioning and operational safety of the instrument can only be ensured if the user observes the usual safety precautions as well as the specific safety guidelines stated in these operating guidelines.

- Before connecting the device to the electrical supply network, please ensure that the operating voltage stated on the power supply corresponds to the mains voltage
- The perfect functioning and operational safety of the instrument can only be maintained under the climatic conditions specified in Chapter 9 "Technical Data" in this operating manual.
- If the instrument is moved from cold to warm surroundings, condense may form and interfere with the functioning of the instrument. In this event, wait until the temperature of the instrument reaches room temperature before putting the instrument back into operation.
- Balancing, maintenance and repair work must only be carried out by a suitable qualified technician, trained by us.
- Especially in the case of any damage to current-carrying parts, such as the power supply cable or the power supply itself, the device must be taken out of operation and protected against being put back into operation.
- If there is any reason to assume that the instrument can no longer be employed without a risk, it must be set aside and appropriately marked to prevent further use.
- The safety of the user may be endangered, e. g., if the instrument
    - is visibly damaged;
    - no longer operates as specified;
    - has been stored under adverse conditions for a lengthy period of time;
    - has been damaged in transport
- If you are in doubt, the instrument should be sent back to the manufacturer WPI for repair and maintenance.
- The operator of this measuring instrument must ensure that the following laws and guidelines are observed when using dangerous substances:
    - EEC directives for protective labor legislation;
    - National protective labor legislation;
    - Safety regulations for accident prevention;
    - Safety data-sheets of the chemical manufacturer

The pHOptica micro is not protected against water spray;
The pHOptica micro is not water proof;
The pHOptica micro must not be used under environmental conditions which cause water-condensation in the housing;
The pHOptica micro is sealed;
The pHOptica micro must not be opened;

We explicitly draw your attention to the fact that any damage of the *manufactural* seal will render of all guarantee warranties invalid.

Any internal operations on the unit must be carried out by personal explicitly authorized by WPI and under antistatic conditions.

Needle-type sensors are housed in extremely sharp syringe needles. Avoid injury by handling the needle carefully. Please pay attention to all safety guidelines for safe handling of sharp needles and syringes. Beware of injuring with the needle as well as with the sensor tip. The glass fiber can break if pricked into the skin and can cause inflammation.

The pHOptica micro may only be operated by qualified personal.
This measuring instrument was developed for use in the laboratory. Thus, we must assume that, as a result of their professional training and experience, the operators will know the necessary safety precautions to take when handling chemicals.

Keep the pHOptica micro and the equipment such as PT1000 temperature sensor, power supply and sensors out of the reach of children!

As the manufacturer of the pHOptica micro, we only consider ourselves responsible for safety and performance of the device if

- the device is strictly used according to the instruction manual and the safety guidelines
- the electrical installation of the respective room corresponds to the DIN IEC/VDE standards.

The pHOptica micro and the sensors must not be used in vivo examinations on humans!

The pHOptica micro and the sensors must not be used for human-diagnostic or therapeutically purposes!

3 Description of the pHOptica micro Device

The **pHOptica micro** is a precise single channel **Fiber Optic pH System** for pH microsensors based on quartz-quartz glass-fibers of 140 µm. The small outer dimensions, low power consumption and a robust box make it ready for portable and field use. For operation, a PC/Notebook is required. The **pHOptica micro** is controlled using a comfortable software, which also saves and visualizes the measured values.
The **pHOptica micro** has 2 analog outputs (0-5 V) and one trigger input (TTL) to be connected to a data logger. Analog connectors are BNC connectors.
The analog outputs are programmable to deliver pH, temperature, or the raw values (phase shift or amplitude), and the data are called via computer and RS232 (digital) or using the external trigger input (analog).

pHOptica micro instruments features:

- high precision
- portable (battery power optional)
- analog/digital data output
- external temperature measurement

Extension to a multi-channel multi-analyte system

Using a computer port extender providing multiple RS232 ports, up to eight single devices (OXY MICRO, OXY MINI, pHOptica micro, …) can be connected to one single computer. This multi-instrument set-up offers a highly flexible method to create multi-channel, multi-analyte measuring systems including additional temperature-compensation of each channel.

Front Panel

ELEMENT	DESCRIPTION	FUNCTION
POWER	ON/OFF switch	Switches the device ON and OFF
SENSOR	ST fiber connector	Connect the fiber-optic sensor here.
L1	Control LED	red: instrument off; green: instrument on; orange: stand by;
Temp	Connector for PT 1000 temperature sensor	Connect the PT 1000 temperature sensor for temperature compensated measurements here.

Rear Panel of the pHOptica micro device

Two standard BNC connectors are added for analog output channels 1 and 2, another one for external trigger input.
The electrical specifications of all rear panel connectors are given in technical specification sheet. Please read also the technical notes to avoid mistakes.

ELEMENT	DESCRPTION	FUNCTION
12 VCD	Line adapter for power supply	Connector for 9 - 36 V DC power supply.
RS 232	RS232 interface (male)	Connect the device with a RS232 data cable to your PC/Notebook here.
CH 1	Analog out (channel 1)	Connect the device with external devices, e.g. a data logger
CH 2	Analog out (channel 2)	Connect the device with external devices, e.g. a data logger
EXT TRIG	External trigger input	Connect the device with external devices, e.g. data logger with a trigger output, pulse generator

4 Required Basic Equipment

- pH meter pHOptica micro*
- Software *pHOpt_v1.exe* for pHOptica micro*
- PC / Notebook
  (System requirements:
  Windows 95/98/2000/Millenium/NT 4.0/XP; Pentium processor, at least 133 MHz, 16 MB RAM)
- RS 232 Cable *
- Line adapter (110 - 220 V AC, 12 V DC) *
- Temperature sensor PT 1000*
- Analyte-sensitive microsensor
  The sensors are mounted into different types of housings
- Vessels for calibration solutions

We recommend Schott laboratory bottles with a thread which can be obtained by VWR International (ordering number: 215L1515)

- Laboratory support with clamp, sensor-manipulator

*: scope of supply

5 pH-Sensitive Microsensors: Sensors and Housings

Optical pH Sensors designed for pHOptica micro device are based on the new **D**ual **L**uminophor **R**eference method (see also appendix). They consist of an inert long decay time reference dye and a short decay time indicator dye, which changes its fluorescence intensity due to the pH-value. The measured value, the average decay time, represents the ratio of the two fluorescence intensities. Therefore, the signal is internal referenced and insensitive to fluctuations of the light source.

Dynamic range

Optical pH sensors respond according to the mass acting law, hence, they do not show a linear behavior like potentiometric pH electrodes. This limits their use to a dynamic range of approximately 3 to 4 pH units. On the other hand, the resolution can be very high in the optimal range. Figure 5.1 shows a typical response curve of a pH sensor. Increasing the pH, the signal - in our case the phase angle Φ - decreases. The phase angle Φ can be related to the pH as shown in Figure 5.2. The theoretical aspects are explained more detailed in the appendix.

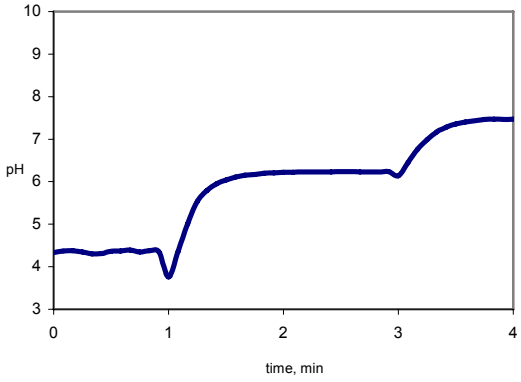


Figure 5.1 Response of pH micro-sensor pH-HP-5.

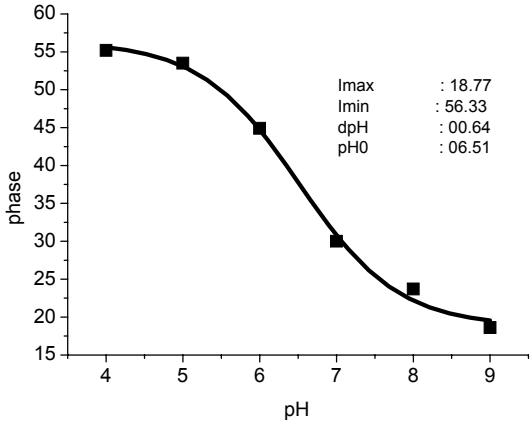


Figure 5.2 Effect of the phase angle of a pH microsensor (pH-HP-5) on different pH values.

The characteristic of a typical sensor are listed below:

Dynamic range: pH 5.5 to 8.5
Resolution: 0.01 pH
Drift due to bleaching: 0.03 per 1000 measuring points
Response time: less then 30 sec

Please note that the given values may differ from your special device and sensoric application.

Resolution

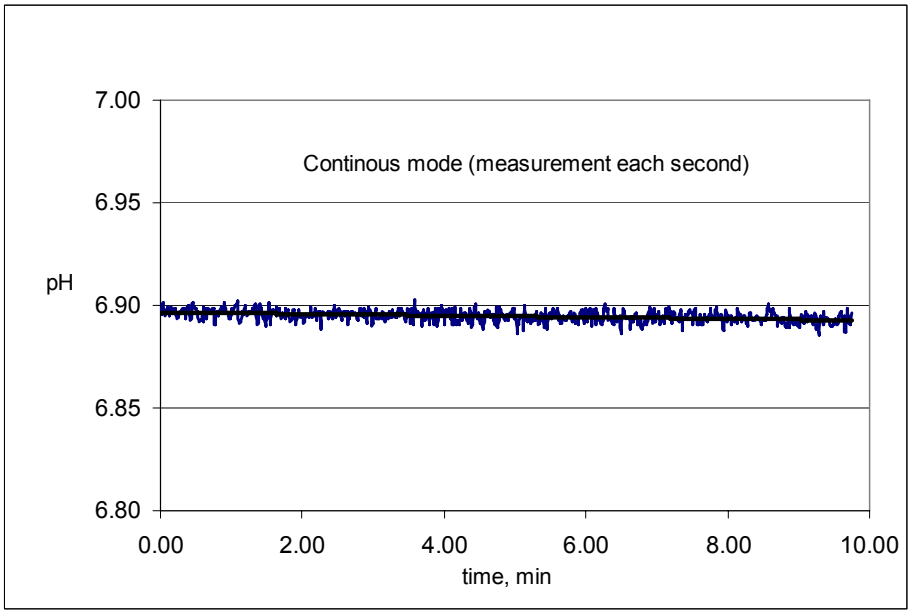


Figure 5.3. Resolution of the pH sensor can be up to 0.01 pH

Cross sensitivities

While pH electrodes are influenced by sulfide, electromagnetic fields or flow velocity the optical pH measurement is interfered by ionic strength. This problem can be overcome by a calibration with buffers of similar ionic strength than the sample.

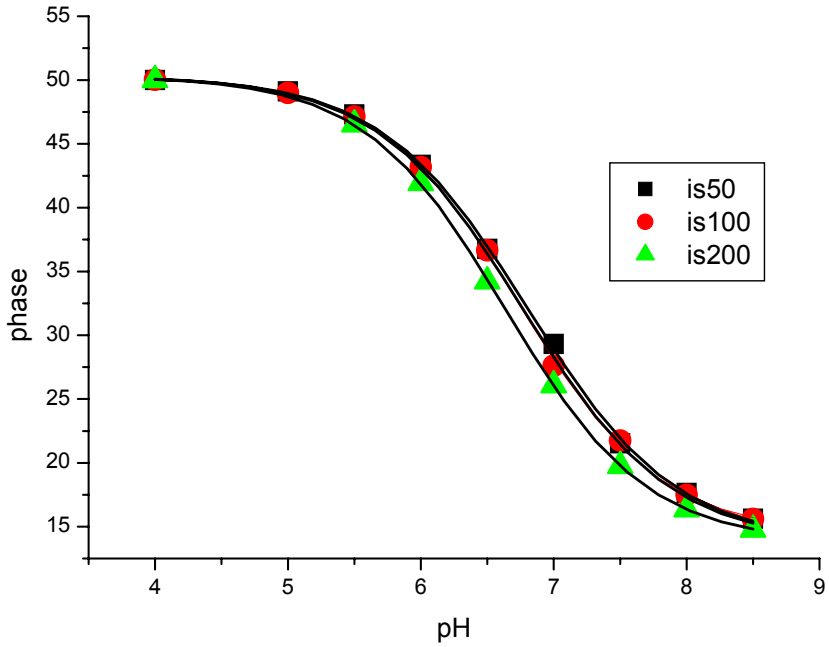


Figure 5.4 Cross-sensitivity to ionic strength – we recommend to calibrate the sensor in pH-adjusted media!

Sensor drift

Like all optical chemo sensors, optical pH-sensors are suspect to photobleaching. Due to this process the measured intensity (amplitude) is decreasing while the phase of the sensor is increasing. This process is quite slow and depends on the used light intensity and the duration of illumination. If you are in doubt whether a measured increase in phase is due to photobleaching or decreasing pH of the sample, please lower the light intensity. If the drift is not getting smaller, your signal drift is due to pH changes in your sample.

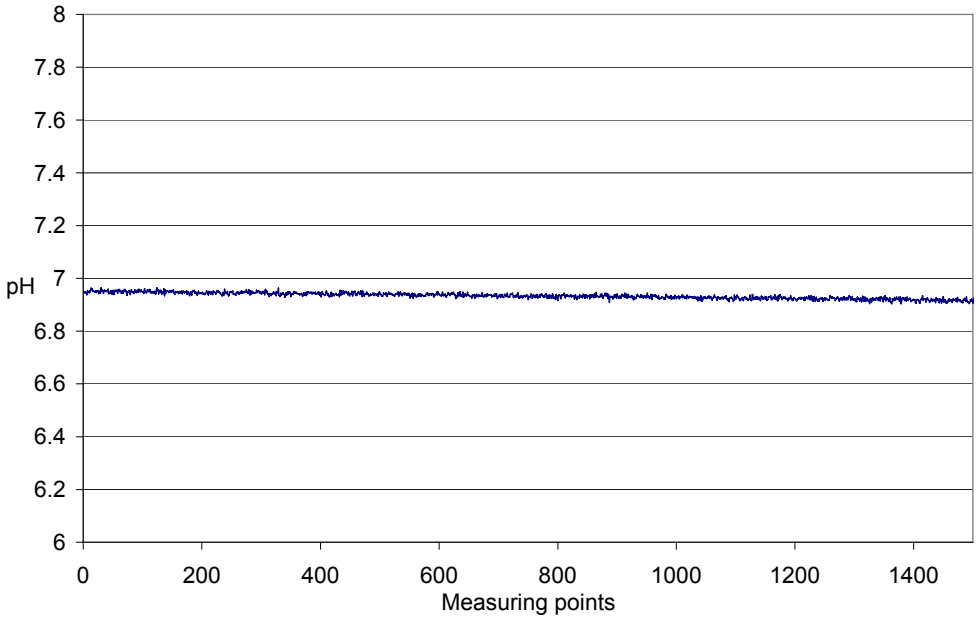


Figure 5.5 Drift due to bleaching: 0.03 per 1000 measuring points

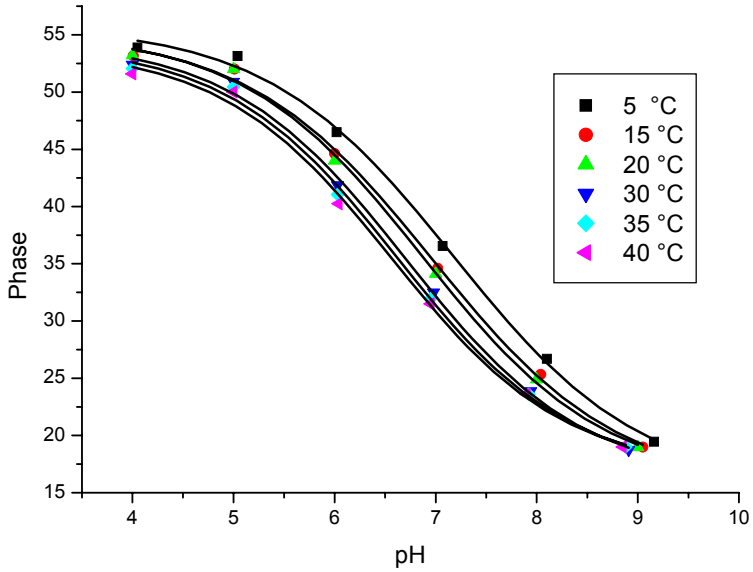
Typical bleaching rates are listed below.

	Sensor Drift due to Bleaching
1000 Measuring points	0.03 pH
1 hour in sec. Mode	0.1 pH
1 d in 1 min Mode	0.05 pH

Please notice, that bleaching rates also depend on the adjusted light intensity of the LED and the individual instrument and sensor.

Temperature dependency

pH Micro sensors display, as any other pH sensors, a distinct temperature sensitivity. Some typical calibration curves at different temperatures are shown below:



To obtain most reliable results we recommend to calibrate at the same temperature as used for measurement. Higher temperature mimics lower pH. At pH 7 a deviation of about 0.08 ph / 5° C occurs.

Limitations

The offered pH sensors for pHOptica micro were specially designed for physiological samples and media. Samples with extremely low ionic strength and low buffer content may be not measured correct. To test whether the sample can be measured correct, try to perform the calibration procedure with a buffer system which is as similar to the sample as possible. If the calibration procedure does not result in a sigmoidal shaped calibration plot, the system is not suited for the sensor. Please contact our service team for special sensors.

The measurement can also be influenced by small, highly fluorescent molecules like fluorescein or rhodamin in the sample.

The sensors do not stand pH above 9 for prolonged time and organic solvents.

Response time

The response time (t_{90}) of the pH sensor is dependent from the diffusion rate of protons through the sensor layer, hence, the response time is dependent from the thickness of the sensor layer and the stirring rate. The typical response (t90) time of a pH-micro sensor is below 30 sec.

5.1 Housings of pH-Sensitive Microsensors

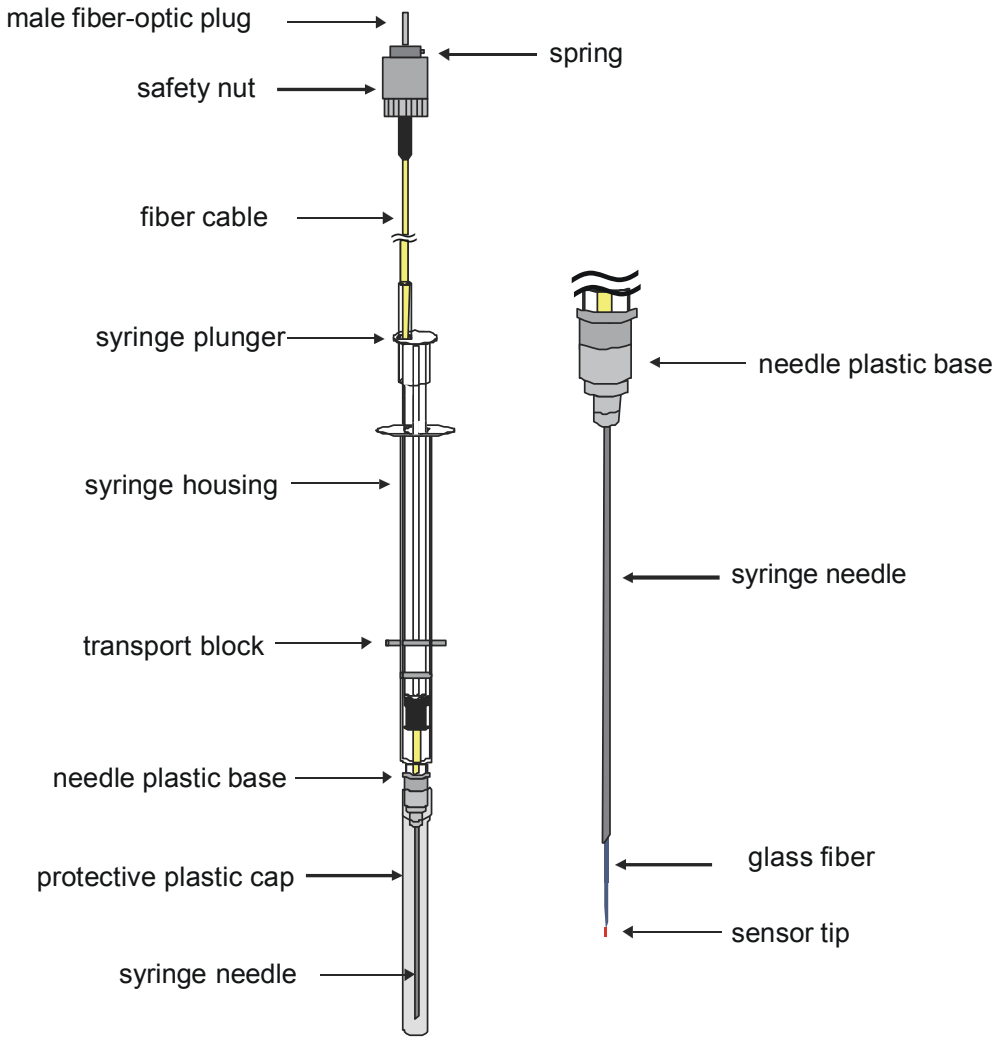
WPI fiber-optic pH microsensors are based on 140 µm silica optical fibers. To protect the small glass fiber tip against breaking, suitable housings and tubings around it, depending on the respective application, were designed.

WPI offers the following standard designs:

5.1.1 Needle-type - pH Micro-Sensor (NTH)

Needle-type pH micro-sensors are miniaturized fiber optic chemical sensors designed for all research applications were a small tip size of ca. 140 µm are necessary. Needle-type pH micro-sensors are ideal for pH profiling in sediment and biofilms with a high spatial resolution. The pH-sensitive tip of the optical fiber is protected inside a stainless steel needle. Such a design is the best for an easy penetration through a septum rubber or any other harsh material as well as secure for transportation. For measurement purposes the sensor must be pushed out from the needle.

Schematic drawing of the NTH (Needle-type) micro-sensor



FEATURES

- High spatial resolution (ca. 140 µm)
- Penetration probe for insertion into semisolids like sediments or biofilms
- Penetration through septa
- Easy to handle
- Different needle size available
- No reference electrode needed
- High temporal resolution
- Insensible towards electrical interferences and magnetic fields

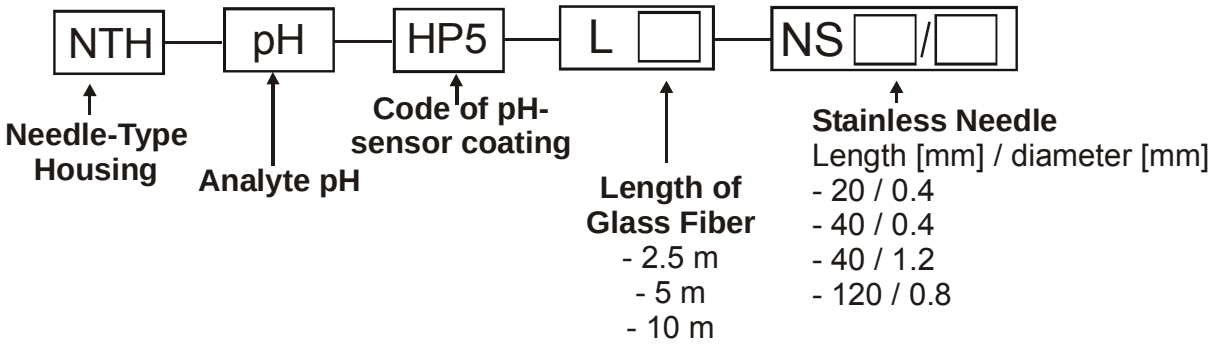
SPECIFICATION

Tip size	140 µm
Measuring range	5 – 9 pH
Response time	30 sec
Resolution	Up to 0.01 pH

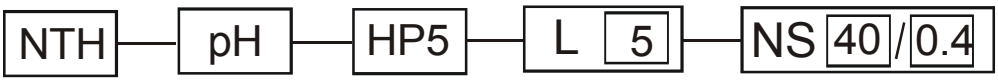
Drift (per 1000 Measuring points)	0.03 pH
Temperature Range	from 5 °C to + 50 °C
Cross-Sensitivity	Optical pH sensors display slight cross sensitivity to ionic strength (salinity) and towards small fluorescent molecules like dissolved indicator dyes.

ORDER

The NTH (Needle-type) pH sensors are offered with options to be specified in the purchase order form. The order code key shown below (see also the example) defines the parameters. Please, choose the parameters that best meet Your requirements.



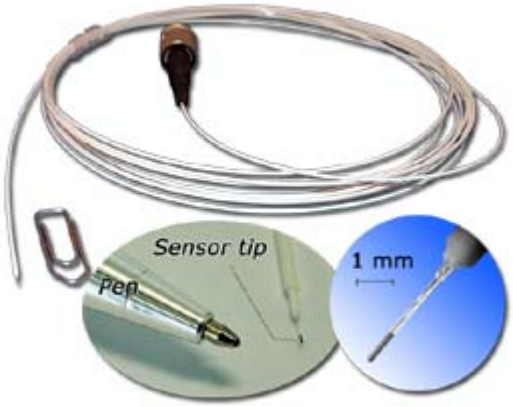
EXAMPLE



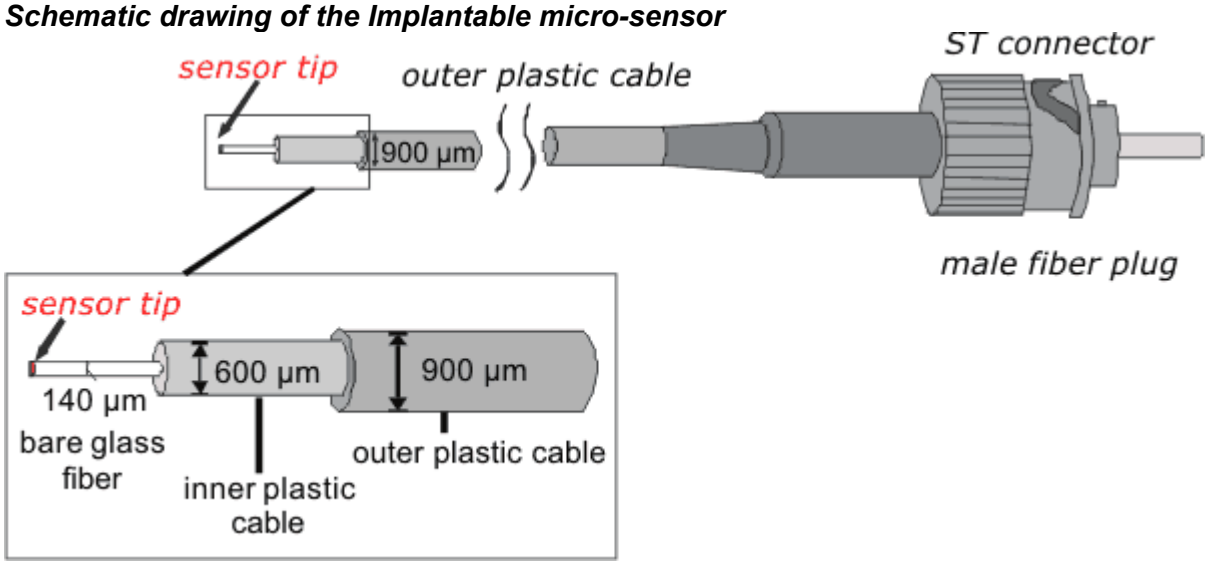
With this code you will order a microsensor mounted in a needle-type housing (***NTH***), with the ***pH*** sensitive coating ***HP5*** with a glass fiber length of 5 m (***L5***) mounted in a stainless needle of 40 mm length and 0.4 mm diameter (***NS 40/0.4***) .

5.1.2 Implantable pH-Micro-Sensor

Implantable pH micro-sensors are the miniaturized fiber optic chemical sensor designed for all research applications were a small tip size of 140 µm are necessary. Implantable pH micro-sensors are ideal for costumer-specific set up. They were applied for implantation in blood circuits and for profiling with a high spatial resolution



Schematic drawing of the Implantable micro-sensor



FEATURES

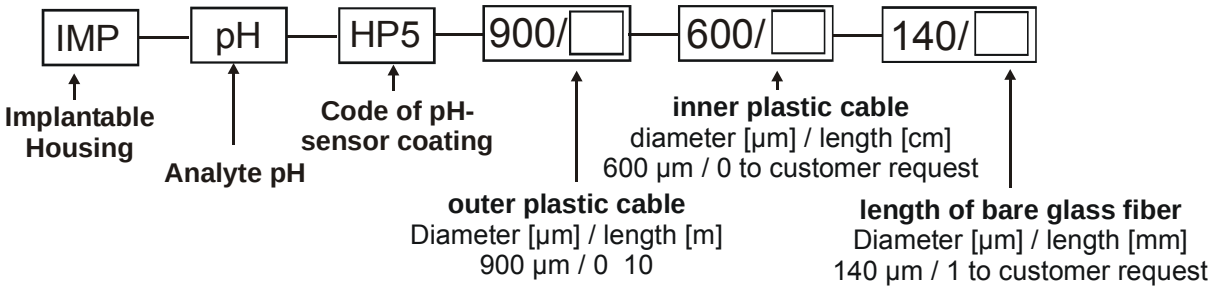
- High spatial resolution (ca. 140 µm)
- Easy to handle
- Most flexible for a wide range of applications
- High temporal resolution
- Insensible towards electrical interferences and magnetic fields

SPECIFICATION

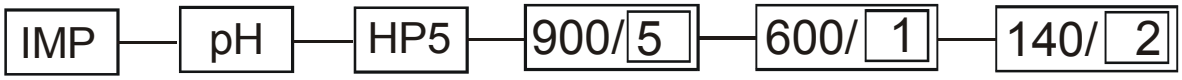
Tip size	140 µm
Measuring range	5 – 9 pH
Response time	30 sec
Resolution	Up to 0.01 pH
Drift (per 1000 Measuring points)	0.03 pH
Temperature Range	from 5 °C to + 50 °C
Cross-Sensitivity	Optical pH sensors display slight cross sensitivity to ionic strength (salinity) and towards small fluorescent molecules like dissolved indicator dyes.

ORDER

The Implantable pH sensors are offered with options to be specified in the purchase order form. The order code key shown below (see also the example) defines the parameters. Please, choose the parameters that best meet Your requirements.



EXAMPLE



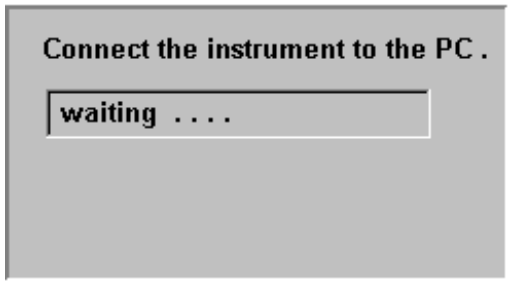
This is the order code for the implantable micro-sensor (***IMP***) with the ***pH*** sensitive coating ***HP5***, with a fiber cable length of 5 m (***900/5***), a cable plastic jacket length of 1 cm (***600/1***), a bare optical fiber length of 2 mm (***140/2***)

6 Description of pHOptica micro Software

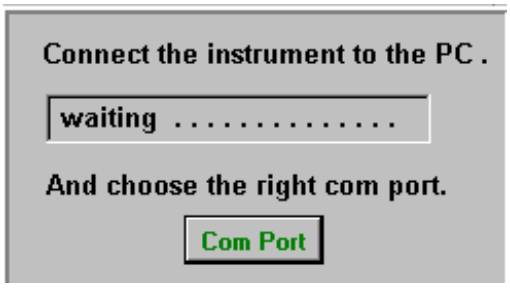
This software is compatible with Windows 95/98/2000/Millenium/NT4.0/XP.

6.1 Software Installation and Starting the Instrument

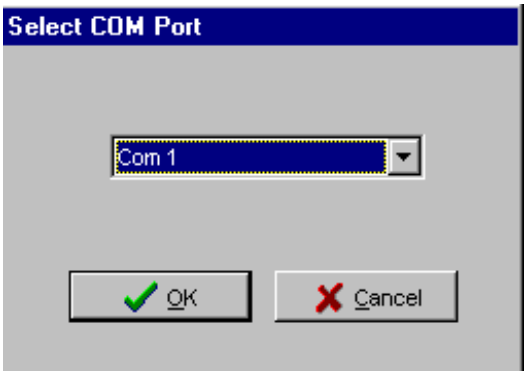
1. Insert the supplied disc/CD into the respective drive. Copy the file *pHOpt_v1.exe* onto your hard disk. (for example, create C:\pHOptica_micro\ *pHOpt_v1.exe*). Additionally, you may create a link (Icon) on your desktop.
2. Connect the pHOptica micro via the supplied serial cable to a serial port of your computer. Tighten the cable with the screws on your computer and on the pHOptica micro.
3. Connect the power supply.
4. Please close all other applications as they may interfere with the software. Start the program *pHOpt_v1.exe* with a double click. The following information window appears:



5. If the right com port is adjusted this information window disappears within a few seconds. If the wrong com port is adjusted you are asked to set the right com port:



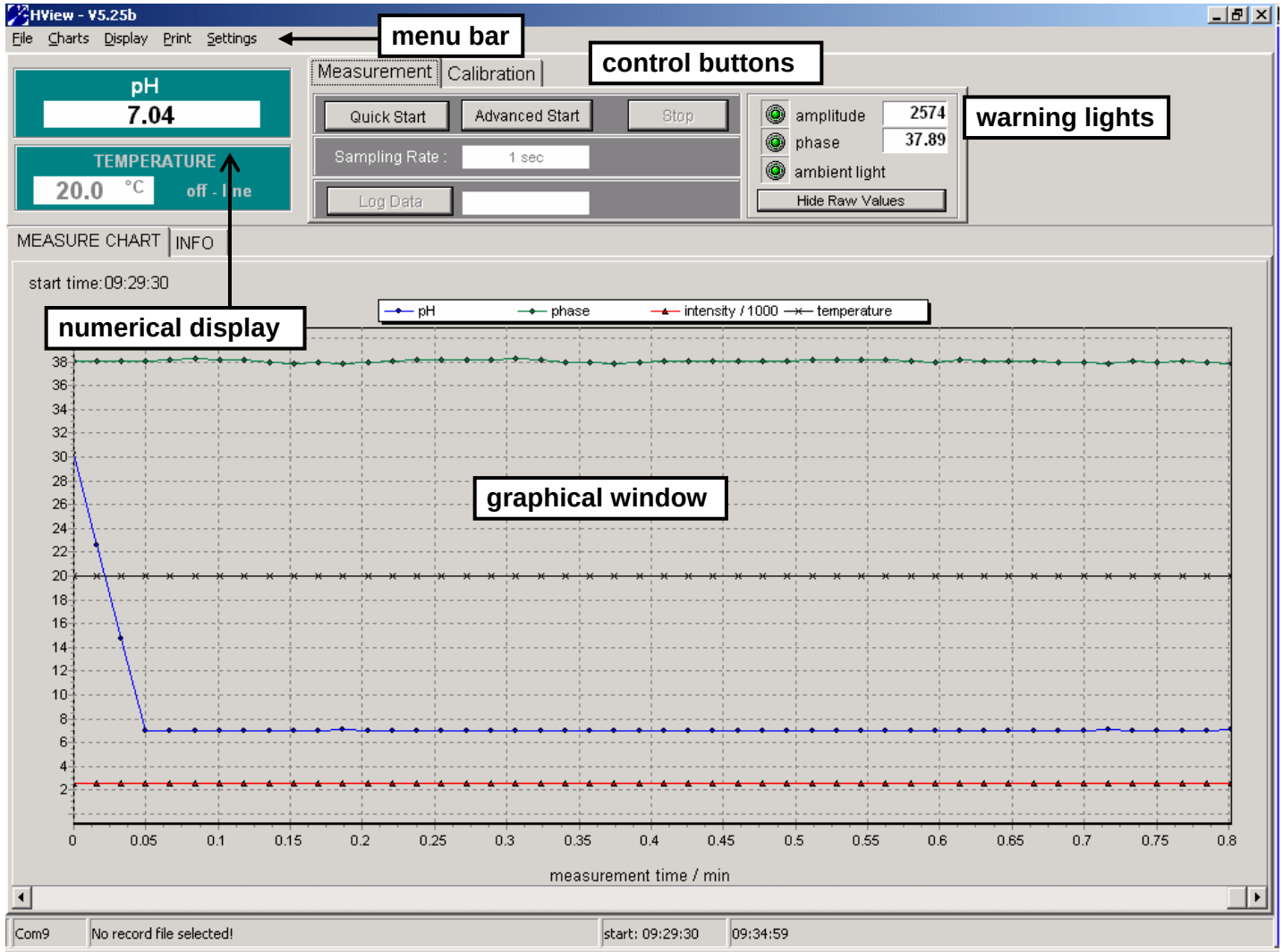
With a right mouse click onto '***com port***' you are able to set the right com port. Please confirm your selection by clicking the '***OK***' button. The information window disappears if the right com port is adjusted.



6.2 Function and Description of the pHOptica micro Program

The window shown below is displayed after starting the software *pHOpt_v1.exe*:
The program has 4 main sections:

1. Menu bar
2. Graphical window
3. Status bar
4. Control bar, divided into numerical display, control buttons and warning lights



6.2.1 Menu Bar

File	*Charts*	*Display*
→ Exit	→ pH	→ Zoom → AutoScaleY1 → Undo Zoom
	→ Temperature	→ Clear Charts
	→ Amplitude	→ Dimensions
	→ Phase	

Print	*Settings*
→ Charts	→ Com Port
	→ Instrument Info
	→ analog settings
	→ LED Intensity
	→ Frequency

File

Exit

Closes the program.

Charts

The respective charts of the measurement can be displayed (√) or hidden

pH:
The measured pH value

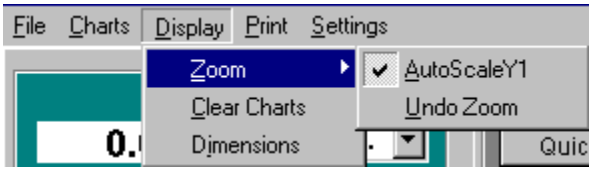
Temperature:
The measured temperature

Amplitude:
The magnitude of the sensor signal

Phase:
Phase angle, the raw data

Display

Zoom:

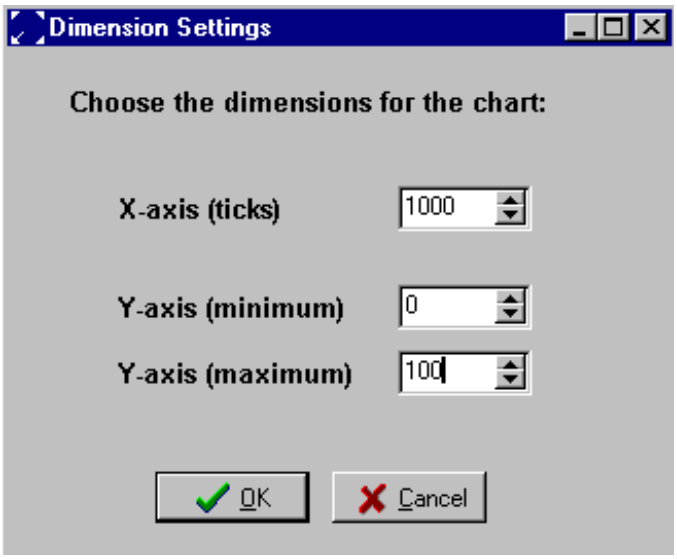


AutoScaleY1 is the default setting. AutoScaleY1 means that the y-axis is scaled automatically.

Undo Zoom: The original display is recovered; see also graphical display

Clear Charts: The graphs shown on the display is cleared.

***Dimensions**:*



You can adjust the number of measurements points on the x-axis shown in the display (maximum number of points are 5000)

Furthermore, you can adjust the micromum and the maximum of the y-axis.

The AutoScaleY1 function is switched off.

Print

Charts: *The charts shown in the display can be printed*

Settings

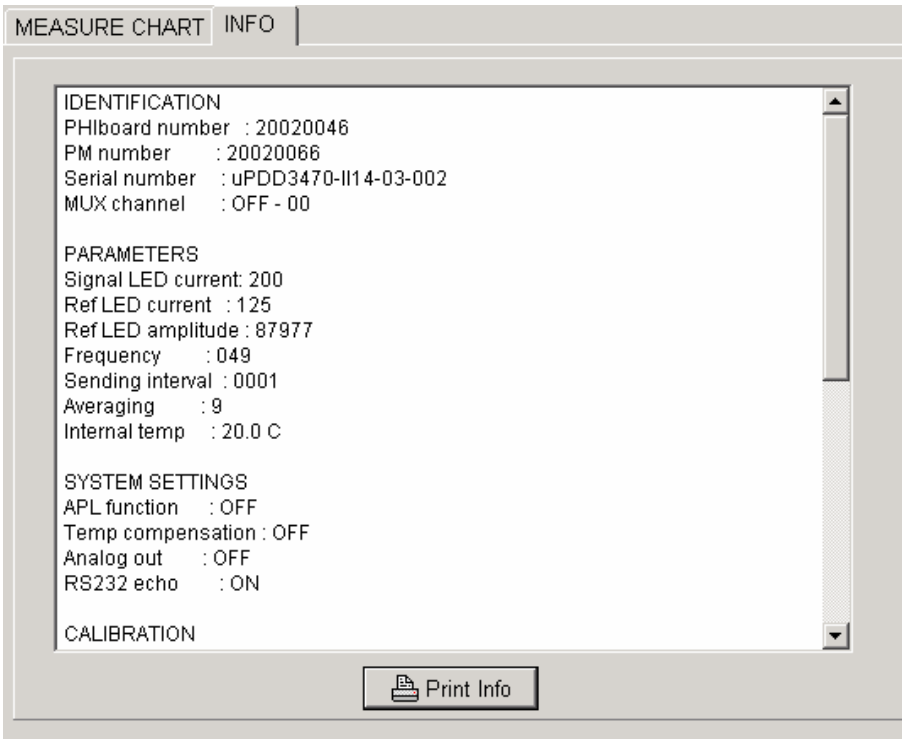
ComPort

The serial comport (com1 – com20) for the serial interface (RS 232) can be chosen in this window. COM 1 is the default setting. If you choose the wrong Com port, the information window '***Connect the instrument to the PC and choose the right com-port***' does not disappear.

Instrument Info:

Here you can find the version of the software and some important settings of the instrument. If you have a problem with the pHOptica micro device, please contact our service team and have the software and instrument information ready.
To change back to the graphical window click the '***Measure Chart***' button.

Instrument Info



Software Info

LED-Intensity

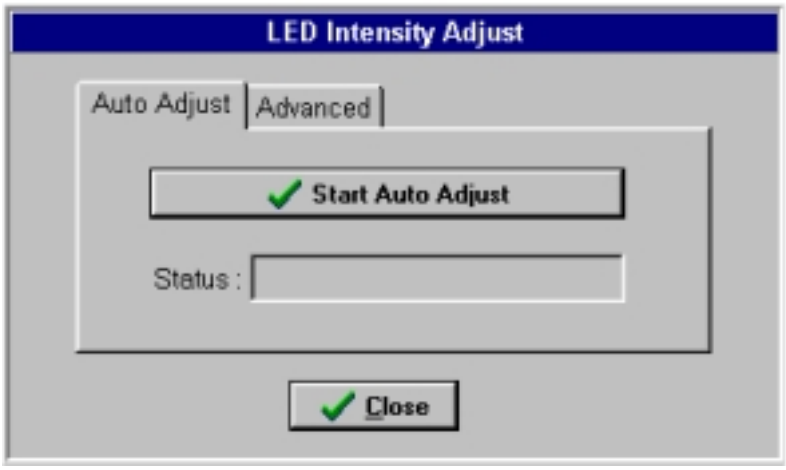
With the current of the LED you can adjust the amount of light illuminating the sensor.

You can choose between an '**Auto Adjust**' of the LED where the pHOptica micro adjusts the optimal LED current itself, or you can select '**Advanced**' where you can adjust the LED current yourself.

If you increase the LED current, the signal amplitude increases, since a higher light density illuminates the sensor. Higher amplitudes lead to more stable values and therefore higher resolution, but also to increased photobleaching.

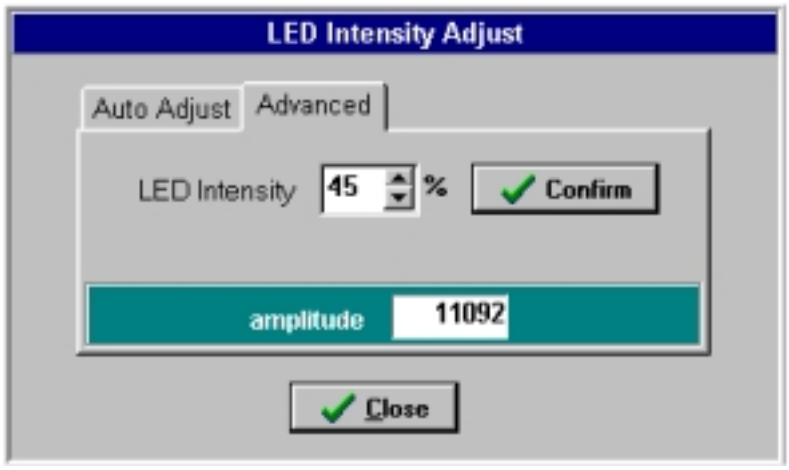
Auto Adjust:

To make the adjustment of the LED intensity automatically, just click the button '**Start Auto Adjust**'. Please check that the Microsensors has been connected to the instrument.



The automatically adjustment of the LED intensity is finished when in the status window the message '**Auto adjustment finished**' appears. Click the '**Close**' button to confirm the settings.

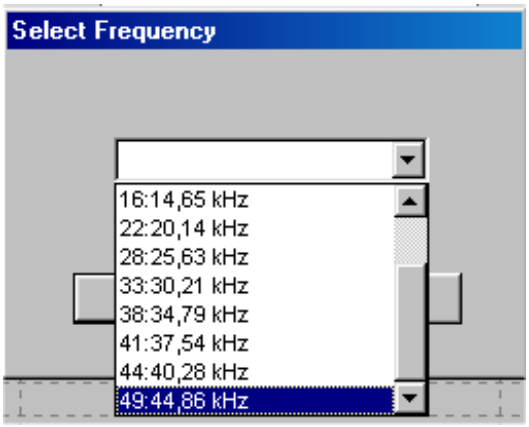
Advanced:
Click the '**Advanced**' button to change the LED current manually. Values between 10 and 100 % are possible. After clicking the 'confirm' button you can see the change of the amplitude in the window below.



Please note:

By increasing the light intensity you increase the amplitude of the sensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

***Frequency* - This option is only for advanced users with special adapted sensors. Please refer to the special advice of our service team.**



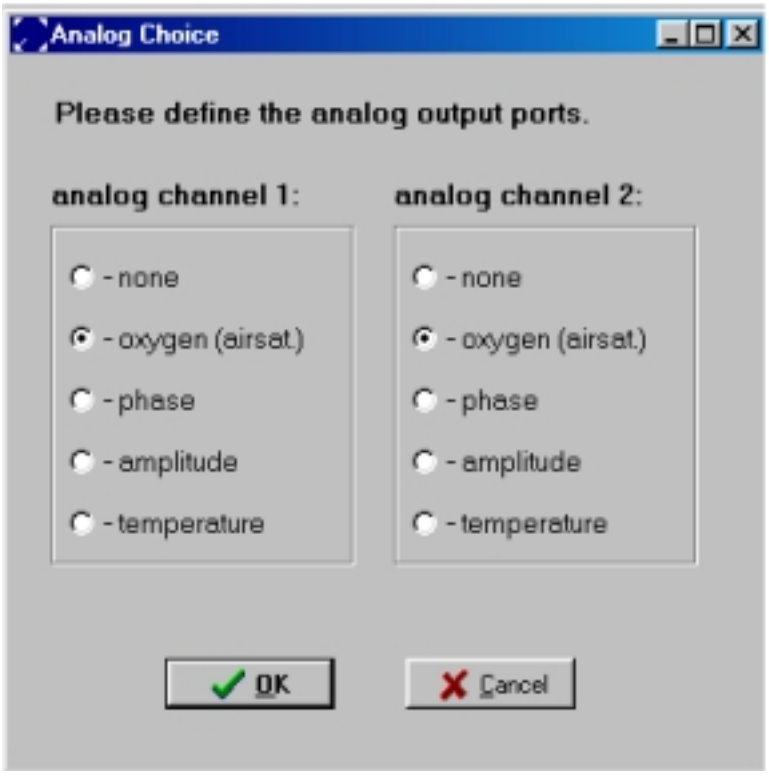
With the menu frequency you can adjust the optimal modulation frequency of your analyte-sensitive indicator dye. Please chose **49: 44.86 kHz** for the pH sensor ***pH-HP5***.
If you change the settings you have to restart the software.

Analog output

Here you can choose which data are exported via the analog output. The pHOptica micro device has two analog outputs and one trigger input. The desired data sources (temperature, amplitude, phase) can be chosen via the dialog box.

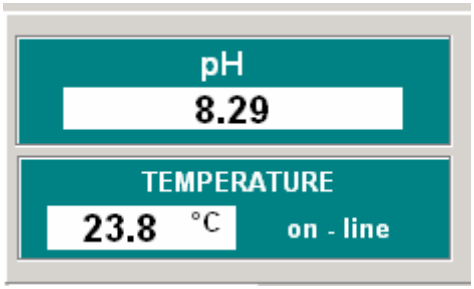
Equivalence coefficient

temperature	1 : 0.1 (e.g. 208mV = 20.8°C)
amplitude	1 : 20 (e.g. 1110mV = 22200 relative units)
phase	1 : 0.025 (e.g. 1100mV = 27.50°)



6.2.2 Control Bar

Numerical display

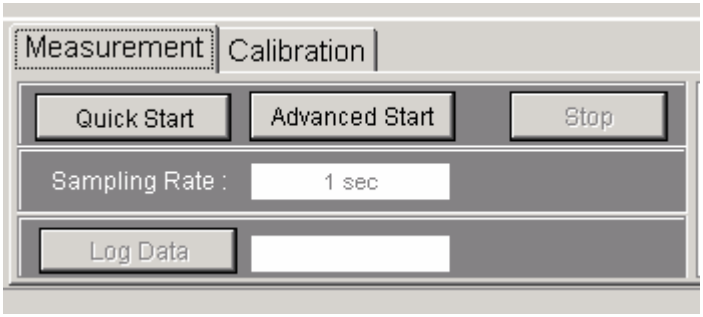


The measured pH is shown in the display window.

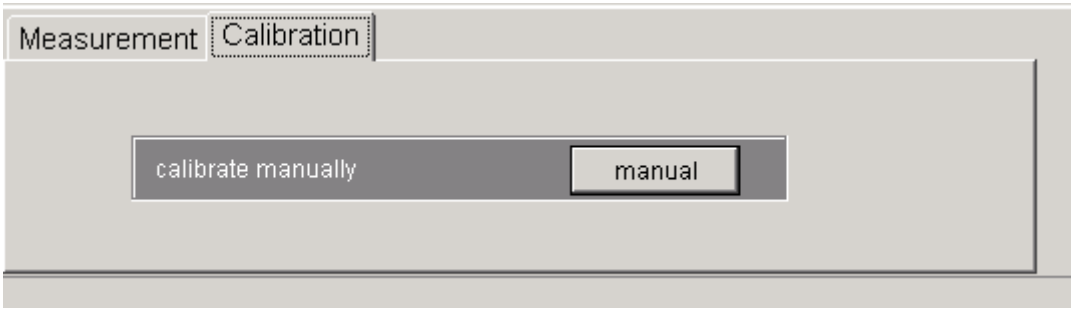
Temperature measurement:
The actual temperature value of the sample (in the case of temperature compensated measurements) is displayed in the temperature window.
If measurement is performed without temperature compensation, the manual inserted temperature is displayed with the hint that temperature measurement is off–line.

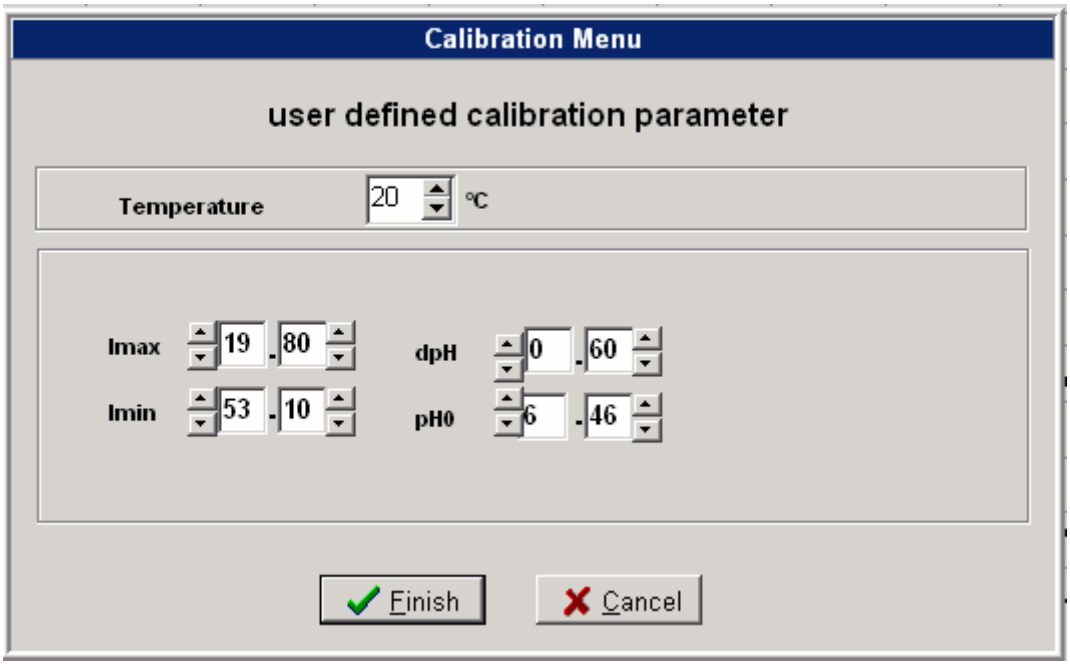
Control buttons:

The way to start a measurement is
(1) Calibration of the sensor by input of calibration values
(2) Start Measurement with Assistant
(3) Log Data



Calibration:





Type in the calibration values. Calibration values are obtained using **pHSolver-v07.exe**.
Please see ***7.1* pHSolver-v07.exe used for calculation of the calibration values** for details

Measurement:
The measurement assistant is opened (default setting).

Quick Start:
The measurement is started. The measurement settings are continuous mode which means that each second a new measurement data is recorded. The measurement is temperature compensated i.e. a temperature sensor has to be connected. If no temperature sensor is connected the following warning window appears.

Click the '**Close**' button if you want to continue the measurement without temperature compensation. The temperature is set to 20 °C by the software. Connect the temperature sensor if you want to perform a temperature compensated measurement.

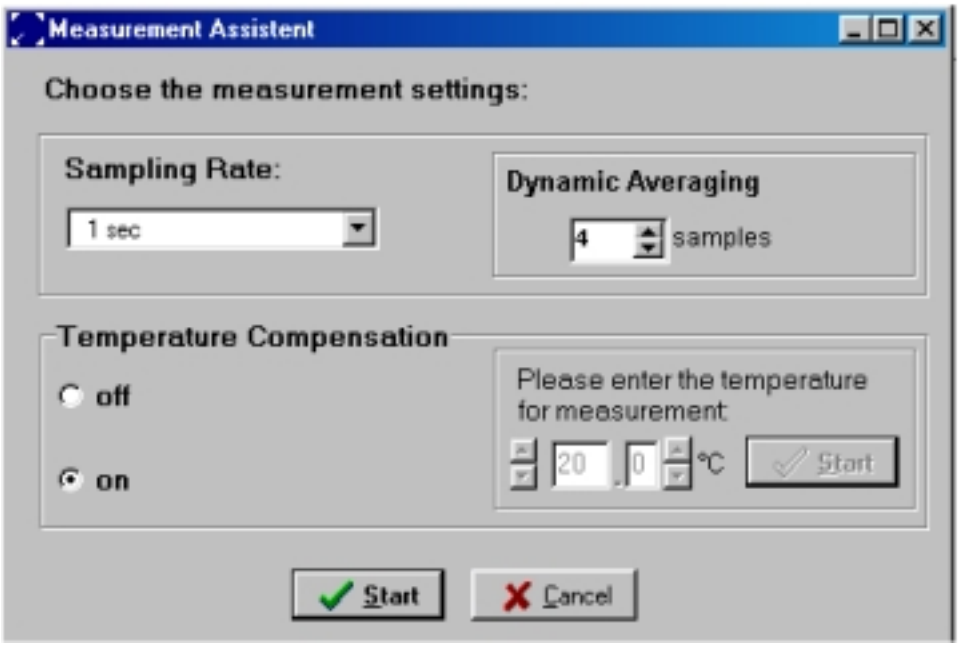
If you want to change the measurement settings click the '**Advanced start**' button.

Please note:

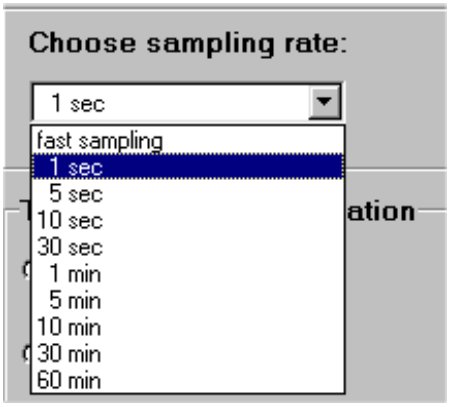
The measurement values are not stored. Click the '**Log Data**' button to store the measurement data.

Advanced Start:

In the '***Advanced Start***' mode it is possible to adjust user-defined measurement settings.



In the '**Sampling Rate**' window you can select the desired measurement mode with a drop-down menu.



By clicking the drop down menu you can choose from '***fast sampling***' (update rate each 250 – 450 ms) to the '***60 min***' mode where each hour a measuring point is recorded.

The speed of recording a measurement point in the '***fast sampling***' mode is about 250 ms when no temperature sensor is connected and the analog output settings are switched off and decreases to about 450 ms when connecting a temperature sensor or activating the analog output channels.

Please note:

The sensor shelf life can be increased using a slower measuring mode since the effect of photo-bleaching is reduced. The illumination light is switched off between sampling. A further advantage using a high measuring mode is that huge amounts of data for long-time measurement can be avoided.

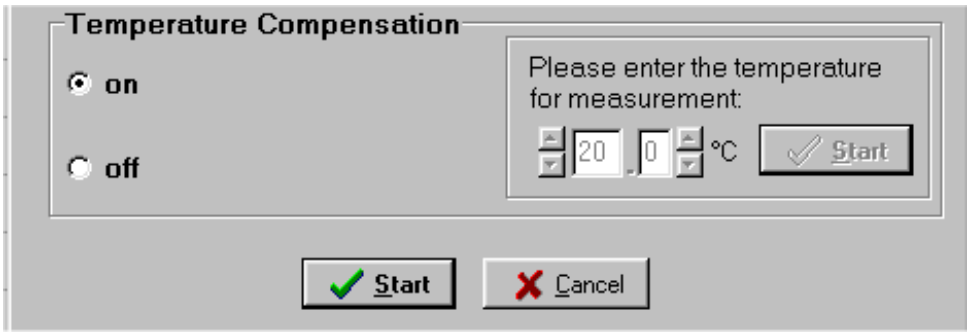
Dynamic averaging

The '***dynamic average***' defines number of averaged measured values. The higher the running average, the longer the time (sampling time) used for averaging. The higher the running average is set, the smoother the measurement signal (maximum 25 samples); The default setting is 4.

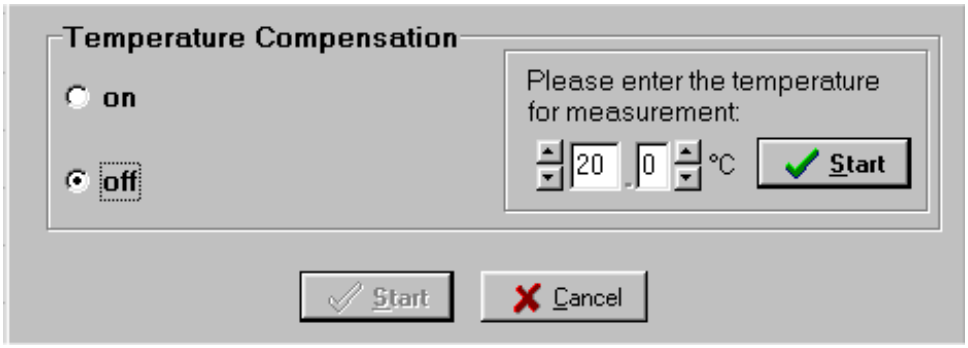
Additional temperature measurements

In the '***temperature compensation***' window you can decide whether you want to measure with or without temperature measurement.

If you want to measure with the additional temperature sensor Pt1000, click the '***on***' button. Please ensure that the temperature sensor Pt1000 is connected to the pHOptica micro, before you click the '***Start***' button to continue. The window where you can enter the temperature manually is disabled.



If you want to measure without temperature compensation, choose the '***off***' button. You will now be requested to enter the temperature of the sample manually. Click the '***Start***' button to start the measurement.

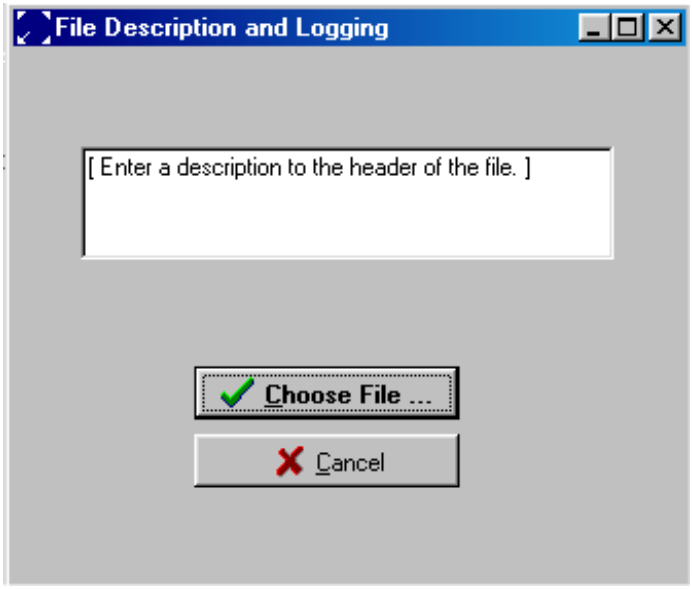


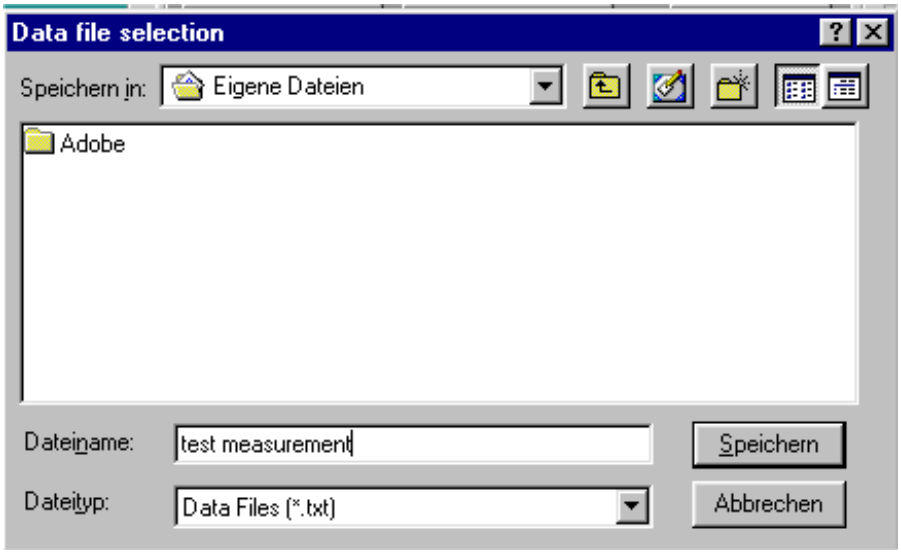
Log Data:

To store the data of your measurement click the '***Log Data***' item. Next to the '***Log Data***' item an information window displays whether the actual measurement is stored to a file (***logging***) or not (***no logging***);

The measurement description which you can enter in the text field '***Enter a description to the header of the file***' is stored in the Ascii File.

By clicking the button '***Choose File***', you can select the location where you want to store the data. Choose as file extension ****.txt***. Click the '***speichern***' button to confirm your settings.





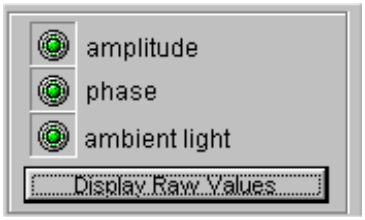
By clicking the '***Stop Log Data***' item you stop data logging which is displayed by the blinking '***no logging***' in the information window next to it.

Stop Measurement

The measurement is ended by a left click on the '***stop***' button in the control bar.

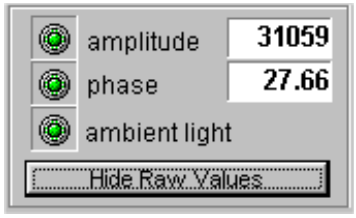
Warning Lights:

At the right bottom of the window you can find the amplitude, phase angle and three warning lights. The warning lights are explained below:



amplitude:	red:	Amplitude is too low, the sensor tip may be damaged or sensor cable may not be connected
	yellow:	Amplitude is critically low, replacement of the sensor is recommended
	green:	amplitude is correct
phase:	red:	phase angle is out of limits
	green:	phase angle is in normal range
ambient light:	red:	background light (e.g. direct sunlight, lamp) is too high. Decrease of false light is recommended
	green:	ratio of sensor signal to false light is acceptable

By clicking the 'Display Raw Values' button, the raw data of phase angle and amplitude are displayed next to the warning lights.



6.2.3 Graphical Window

The respective sensor signal is displayed according to the selection of the 4 control buttons pH, phase, amplitude and temperature (menu chart). The raw values (the phase angle in degrees and the sensor amplitude in mV) can also be displayed by clicking the button ***'Display Raw values'***. The temperature is given in [°C]

Zoom Function:

1. Press the left mouse button and drag from left to right to enlarge a certain area of the graphical window. The graphical window displays the selected data points and is not actualized with new data.
2. Press the left mouse button and drag from right to left to recover the original display, or click the '**Undo Zoom**' button in the *display* menu under z*oom*.

6.2.4 Status Bar

sw1: Displays the serial port which is used for communication of the pHOptica micro device with the PC
sw2: Displays the file name in which the measurement data are stored. „No storage file selected“ is displayed if no file was selected (no data storage).
sw3: Displays the start time of the measurement
sw4: Displays the actual time

6.3 Subsequent Data Handling

In the head of the ASCII file, you find the **description** of your measurement which you have entered by storing the file.
Below you find the ‘***instrument info***’ containing all important settings of the instrument and firmware and the calibration values and the date of the calibration
.
The ‘***software info***’ below contains the version number of the pHOptica micro software, date and time of the performed measurement. If there is a problem with the pHOptica micro device, please contact our service team and have the software and instrument information ready.
Below, you find the ‘***measure mode settings***’ containing the *dynamic averaging*, and the *measuring mode*.

The following rows, separated by semicolons, list the measuring data. The first two rows contain the **date** and **time**, the third the **log-time** in minutes. The pH values are stored in the fourth row. The raw data - **phase angle** in [°] and the **amplitude** in [mV] - are stored in the fifth and sixth row, respectively. The seventh row contains the **temperature** in °C measured by PT1000 temperature sensor. Raw data can be used for user defined recalculations according to the formulas and tables of the delivered Excel sheet

The eigthth raw of the Ascii file displays possible error messages.

***** DESCRIPTION **********
pHOptica micro sample#1

***** INSTRUMENT INFO *******
IDENTIFICATION
PHIboard number : 20030007
PM number : 20030110
Serial number : pH-1-micro-AOT-03-007
MUX channel : OFF - 00

PARAMETERS
Signal LED current: 050
Ref LED current : 058
Ref LED amplitude : 88397
Frequency : 049
Sending interval : 0001
Averaging : 5
Internal temp : 26.8 C

SYSTEM SETTINGS
APL function : OFF
Temp compensation : ON - ch a
Analog out : chA t chB t
RS232 echo : ON

CALIBRATION
Sensor type : 1
Imax : 22.00
Imin : 54.00
dpH : 00.66
pH0 : 07.00
Date (ddmmyy) : 241003
Pressure (mBar) : 0020

FIRMWARE
Code v1.088PH IAP: 07/31/03, 10:45:01
Xilinx built : 20/08/02 (MM/DD/YY)
Reset condition : SLEEP

***** SOFTWARE INFO *********
pHOpt_v1 - 01/2004
24.10.2005
10:43:16

******MEASURE MODE SETTINGS**
Dynamic Averaging 4
measure mode 1 sec

start time 10:37:44

date(DD/MM/YY)	time/hh:mm:ss	logtime/min	pH	phase/°	amp	temp/°C	ErrorMessage
24.10.2005	10:43:16	0	5.09	52.3	3464	21.5	E0
24.10.2005	10:43:17	0.017	5.075	52.34	3475	21.5	E0
24.10.2005	10:43:19	0.034	5.068	52.35	3487	21.5	E0
24.10.2005	10:43:20	0.051	5.08	52.32	3461	21.5	E0
24.10.2005	10:43:21	0.068	5.08	52.32	3477	21.5	E0
24.10.2005	10:43:22	0.086	5.07	52.35	3494	21.5	E0
24.10.2005	10:43:23	0.103	5.105	52.27	3479	21.5	E0
24.10.2005	10:43:24	0.12	5.105	52.26	3468	21.5	E0
24.10.2005	10:43:25	0.137	5.085	52.31	3454	21.5	E0
24.10.2005	10:43:26	0.154	5.098	52.28	3464	21.5	E0
24.10.2005	10:43:27	0.171	5.093	52.29	3468	21.5	E0
24.10.2005	10:43:28	0.188	5.128	52.2	3481	21.5	E0
24.10.2005	10:43:29	0.205	5.12	52.22	3465	21.5	E0

7 Calibration and Measurement

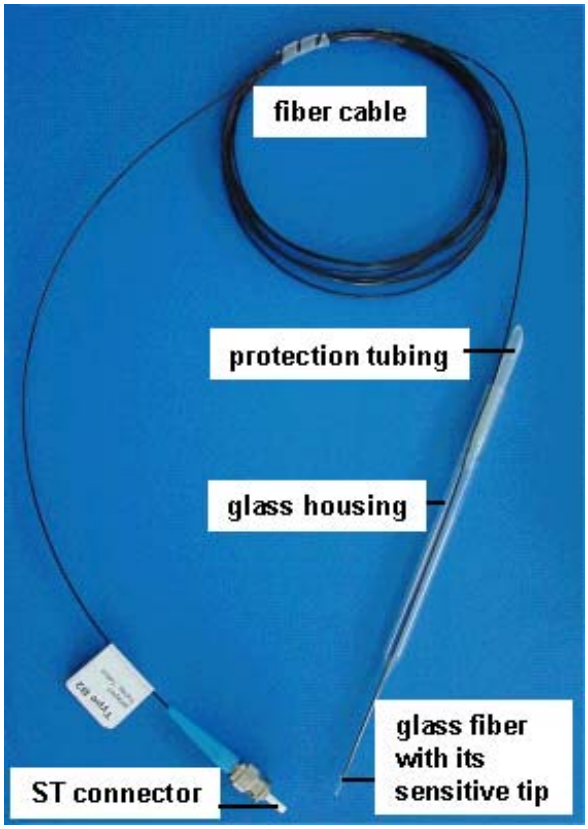
Calibration of the sensors is recommended before each measurement. Each calibration is only valid for the corresponding sensor and should be repeated before beginning a new measurement. Especially, after longer measurements (more than 1000 measuring points) the sensor should be re-calibrated.

7.1 Buffers for Calibration

For the calibration of pH Micro-sensors at least 4 different buffers are needed. To obtain best results a similar composition as the measured sample is recommended. For example, for measurement in cell culture media calibration in buffers with an ionic strength of 140 mM and a phenol red concentration of 15 mg/l is ideal. Please notice, that the pH range of the calibration should exceed the pH range of the measurement – e.g. it is not favorable to calibrate with buffers of pH 4.0, 5.0, 5.5, and 6.0 and to measure about pH 7. Calibration is only correct for interpolation, not for extrapolation.

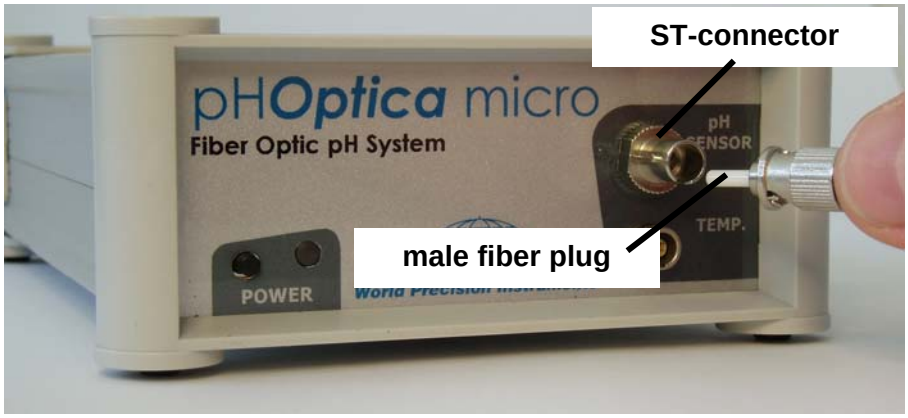
7.2 Mounting the Implantable Microsensors

1. Remove the microsensor carefully from the protective cover. The microsensor is protected with a glass housing during the transport.
2. Fix the glass housing microsensor with a clip to a laboratory support or a similar stable construction.



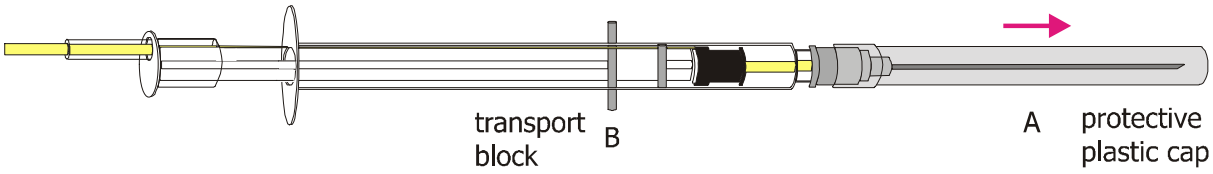
We strongly advise you not to handle with microsensors without the support - especially when the sensor tip is extended.

3. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the pHOptica micro device. The female fiber-plug of the pHOptica micro has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

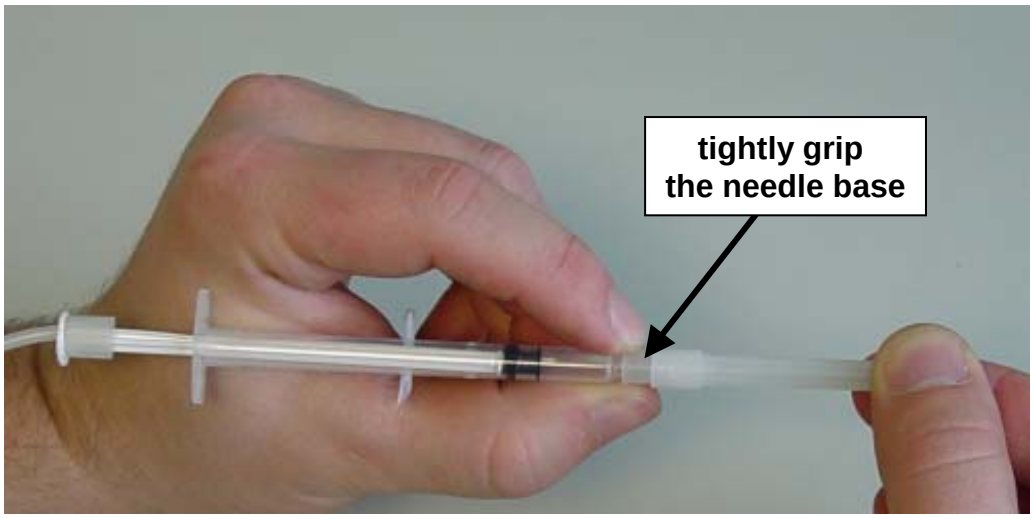


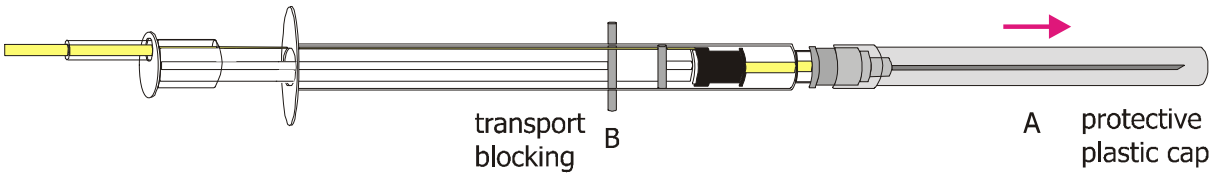
7.3 Mounting the Needle-Type Microsensors

1. Remove the microsensor carefully from the protective cover. The needle-type microsensor is housed in 0.4 x 40 mm syringe needle mounted to a 1 mL plastic syringe housing with integrated PUSH & PULL - IN & OUT mechanism. The syringe needle is protected with a protective plastic cap (**A**).



2. Carefully remove the protective plastic cap (**A**) covering the syringe needle.
When doing so, grip the plastic base of the needle tightly. The syringe needle ***must not*** be removed from the syringe housing. Work carefully!



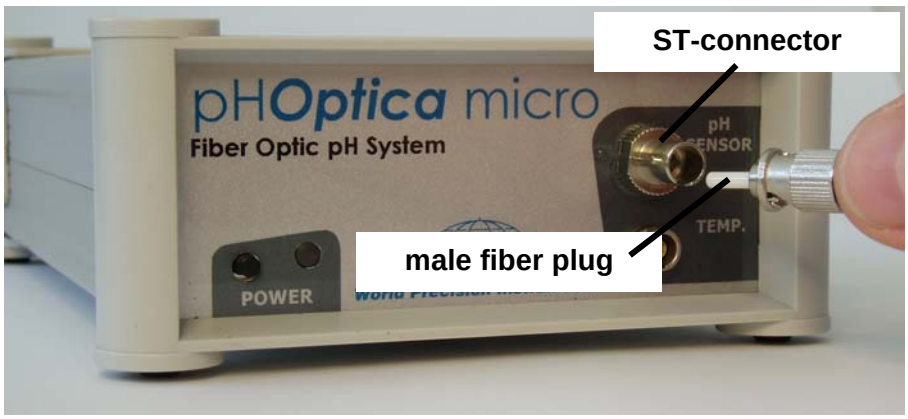


3. Fix the microsensor with a clip to a laboratory support or a similar stable construction.

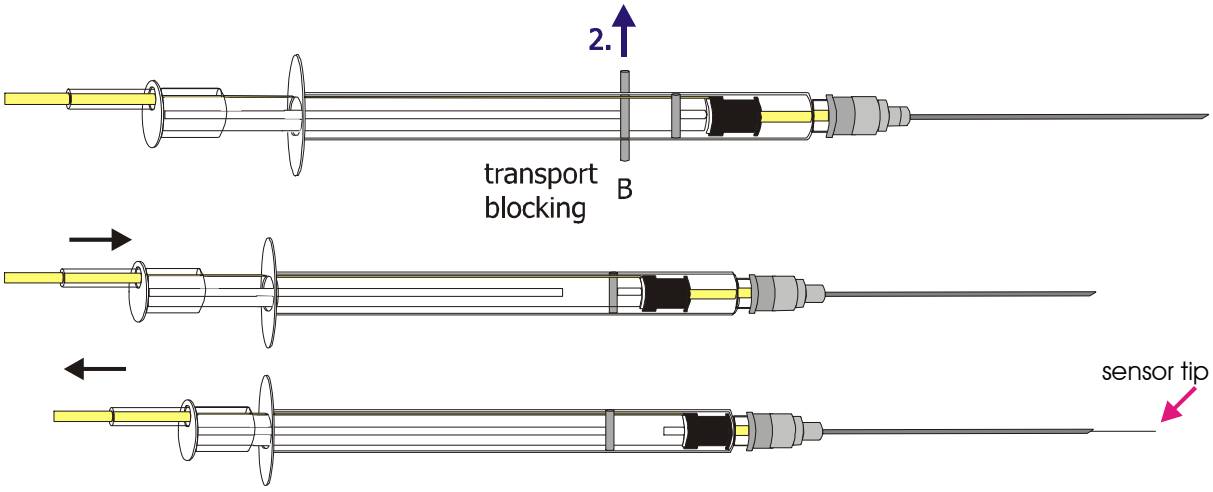
We expressly warn you not to handle with microsensors without the support - especially when the sensor tip is extended.

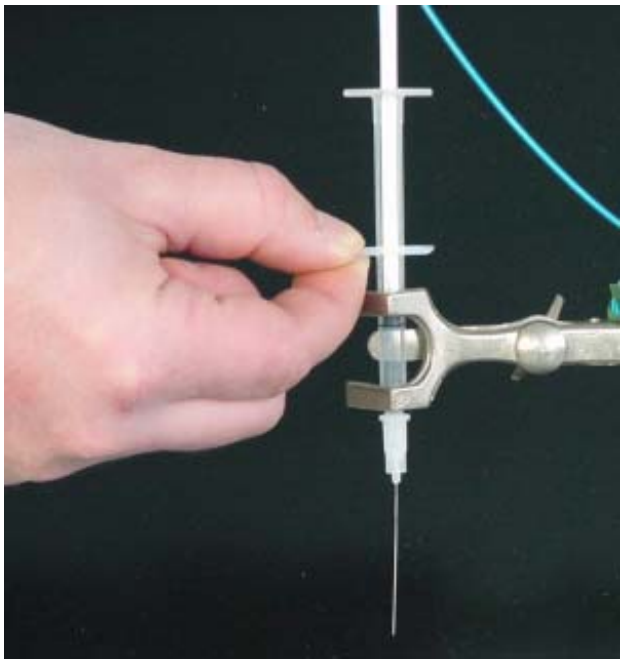
4. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the pHOptica micro device. The female fiber-plug of the pHOptica micro has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety

nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.



5. The glass fiber with its sensing tip is prevented from slipping using a transport block (**B**). Remove the transport block from the hole in the syringe housing. Now it is possible to retract or extend the glass fiber with its sensor tip by pushing or pulling the plunger. Before pushing out the sensor tip, make sure that you have removed the protective plastic cap and have some space in front of the syringe needle.





WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK

7.4 pHSolver-v07.exe used for calculation of the calibration values

To receive pH values it is necessary to determine calibration values of the sensor. This can be done by the use of pHSolver-v07.exe. Other fitting programs (e.g. Origin or MathLab) which can handle the Boltzman equation can be used too.

The delivered File ***pHSolver-v07.exe*** does all the calculations. It calculates a Boltzman curve fit for the measured values.

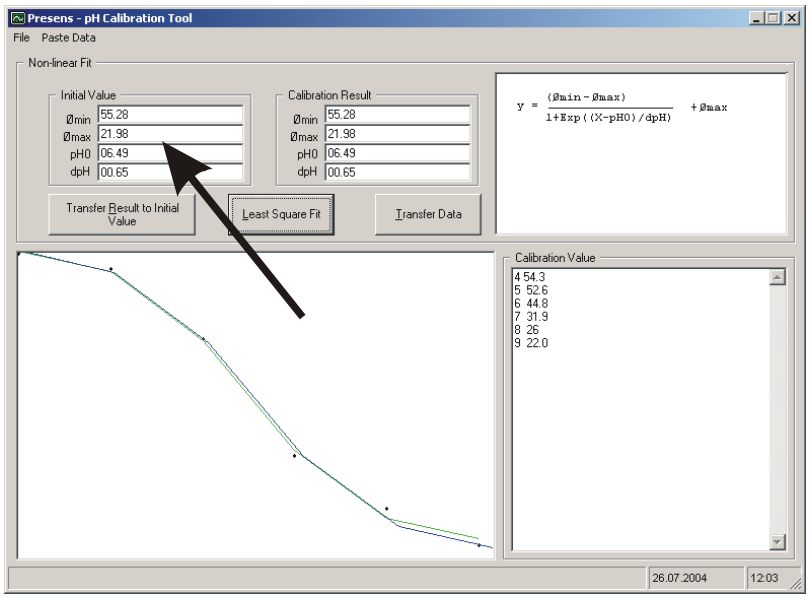
Ensure that dot instead of comma is used as decimal separation (US-Standard)

1) Copy the files libraryfiles.exe and pHSolver-v07.exe to a folder named pH-solver.

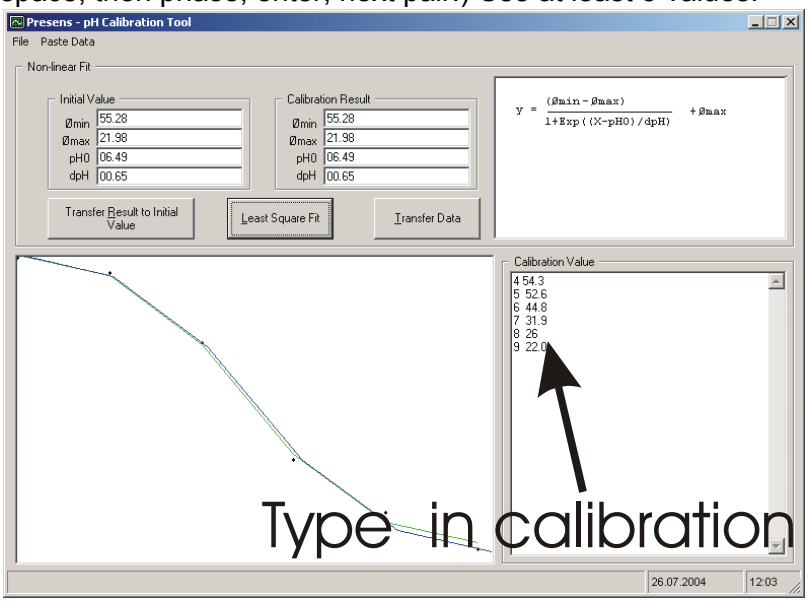
2) Start libraryfiles.exe by doubleclick and follow the instructions. If the software asks you if an existing file should be overwritten, please click on NO.

3) Start the software pHsolver-v07 by double click.

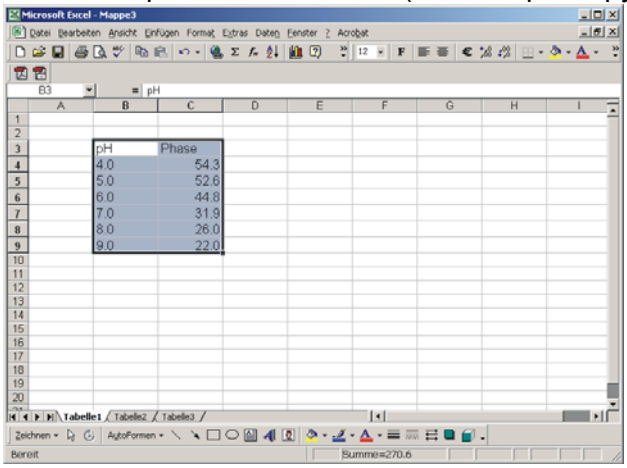
4) Type in the calibration values of the sensor data sheet into the respective "inital value" fields.



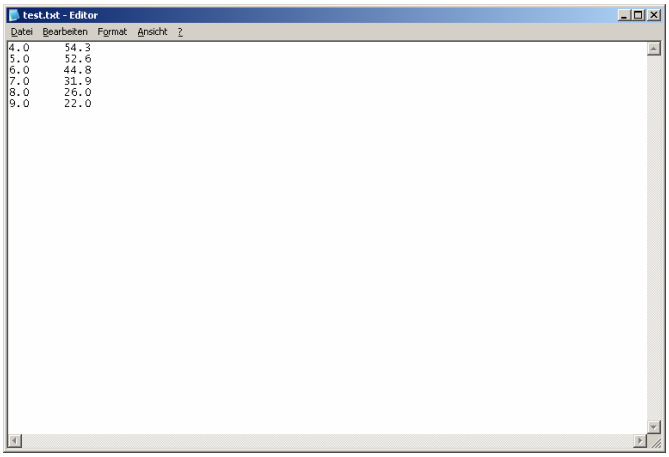
5) Type in the pH-phase value pairs of your calibration (first pH value of the buffer, then space, then phase, enter, next pair.) Use at least 5 values.



Or use the paste data function (for example copy a set of data of a excel sheet).



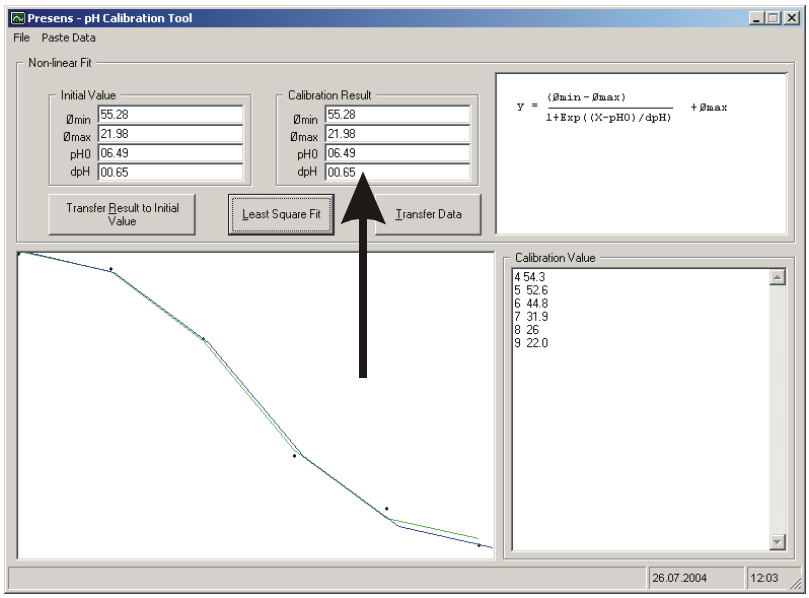
Or open a data file containing the data in the format:



6) Click on "least square fit" and you will get the set of calibration data in the calibration result area. You can store the data in a file by pressing “transfer data”. If the "initial guess" is far from the real data, the software may ask you to try again - please just click on yes, the software will find a new guess on its own.

7.5 Calibration/Measurement of a pH Microsensors

1. Connect the pHOptica micro via the RS232 cable to your computer.
2. Switch on the pHOptica micro and connect the sensor as shown in Chapters 7.2 to 7.3 depending on the sensor in use.
3. Start the pHOptica micro software on your computer and start the measurement (see chapter 6.2.2).
4. Calibrate the pH sensor, pH-sensitive foil with the calibration solutions (e.g. buffer solutions of pH 5, pH 6, pH 7, and pH 8). To minimize the response time, slightly stir the buffer solution. Please ensure that the sensor is dipped in the buffer solution.
5. Wait about 3 minutes until the phase angle belonging the respective buffer is constant (the variation of the phase angle should be smaller than $\pm$ 0.1°)
6. Wash the sensor tip with distilled water to clean it from respective buffer solution before taking the next calibration value.
7. Open the file pHSolver-v07.exe and enter the pH-values of the used buffer (i.e. 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) and the corresponding phase values in the calibration value window (see above).
8. Press the "least square fit” button.
9. The calibration results $phase_{max}$, $phase_{min}$, dpH, pH_0 are shown and the calibration curve is displayed.



10. Wash the sensor with distilled water to clean it from buffer components.

7.6 Some Advice for Correct Measurement

7.6.1 Signal drift due to photo-decomposition

pH – sensor-sensors are suspect to photobleaching which results in a drift of the sensor to lower pH. Photobleaching is higher at basic pH. Photobleaching depends at the amount of light. Therefore you should reduce the amount of light. The pHOptica micro device offers two ways of reducing the amount of light.

First, you can lower the amount of measuring points in the case of long term measurements by choosing the second or minute mode in the measuring assistant (see chapter 6.2.2. **Control Bar**, ***Advanced Measurement***)

Second, you can lower the LED current:
Choose ***LED-Intensity*** in the *Settings* menu of the menu bar.

With the current of the LED you can adjust the amount of light illuminating the sensor spot. By increasing the light intensity you increase the amplitude of the sensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

Bleaching always mimic lower pH. For long term measurements the drift can be estimated by the amount of measurement points and LED intensity. The sensor should be recalibrated after the measurement and the calibration values compared to the initial values to obtain maximum accuracy.

Please notice, that the noise (and therefore the resolution) of the signal depends on the amplitude of the sensor. Lowering the LED current will lead to lower photobleaching but increased noise of the sensor. The best resolution is achieved if the amplitude of the sensor is more then 5000. An amplitude of less then 2000 will result in a high noise of up to 0.1 pH.

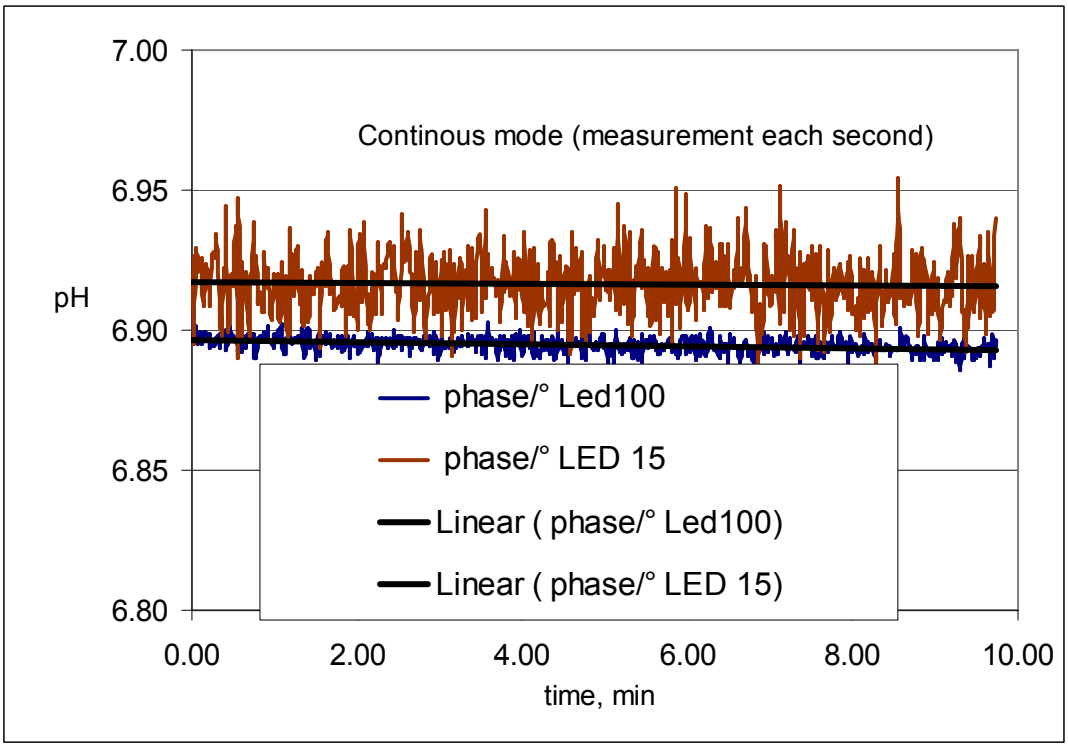


Figure 7.3 Phase resolution illumination the sensor tip with 15% and with 100 % LED current, respectively.

7.6.2 Performance proof

If you want to prove the performance during the past measurement, please check the calibration buffers by inserting the sensor tip in the buffer solution pH 6 and pH 7 when you have finished your measurement. If the device displays the correct pH values, the sensor worked perfectly during the whole measurement.

If you are in doubt whether the sensor has to be changed, please follow this procedure:

1) Expose the sensor to pH 4 – Buffer solution (e.g. Certipur Buffersolution pH 4, Merck, **109435**) and adjust intensity to a value of more then 2000 (ideally more then 10000) by adjusting the LED current (see page 20). If this is not possible and the fiber cable and connection to the sensor is OK, change the sensor. The measured phase must be between 60 and 48 ° - otherwise check frequency (must be 049) or change sensor.
2) Expose the sensor to pH 9 – Buffer solution (e.g. Certipur Buffersolution pH 9, Merck, **109461**). The measured phase must be between 35 and 10° - otherwise change sensor.

8 General Instructions:

8.1 Warm-Up Time

The warm up time of the electronic and opto-electronic components of the pHOptica micro is 5 min. Afterwards stable measuring values are obtained.

8.2 Maintenance

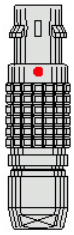
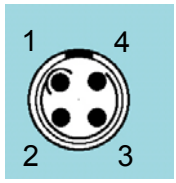
The instrument is maintenance-free.
The housing should be cleaned only with a moist cloth. Avoid any moisture entering the housing! Never use benzine, acetone, alcohol or other organic solvents.
The ST-fiber connector of the sensor can be cleaned only with lint-free cloth. The sensor tip may be rinsed only with distilled water. Please ensure, that no sample residues are inside the syringe needle. If necessary, rinse the glass-fiber with distilled water.

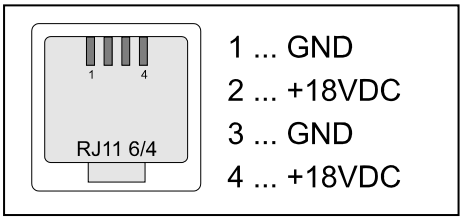
9 Technical Data

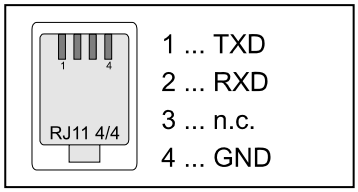
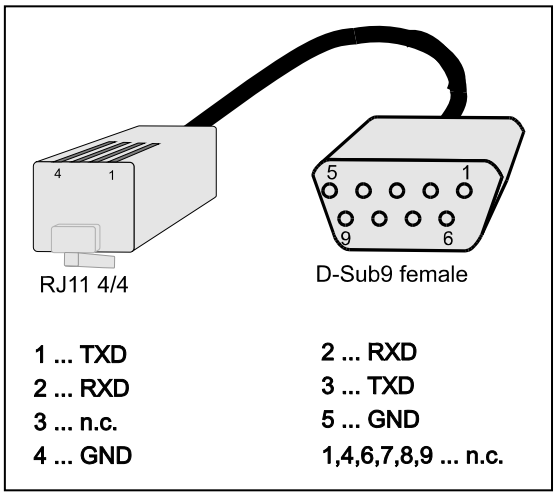
9.1 General Data

MODES	
pH	range: 4.5 – 8.5 resolution: 0.01 pH units
Electrical temperature sensor (Pt 1000)	range: 0 - 50 °C resolution: ± 0.5 °C accuracy: ± 2° C

OPTICAL OUTPUT / INPUT	
Optical connector	ST compatible, Core/Center 100/140
Channels	1
Wavelength	470 nm

TEMPERATURE SENSOR INPUT	
Lemo Connector Size 00	Connector for Pt-1000 temperature sensor   **1 ... PT1000-1** **2 ... n.c.** **3 ... n.c.** **4 ... PT1000-2**

DC INPUT	DC-Range : 12 V/1250mA up to 18V/900mA 

DIGITAL OUTPUT	
communication protocoll	serial interface RS232 19200 Baud, Databits 8, Stoppbits 1, Parity none, Handshake none
instrument output:	on RJ11 4/4 plug 
Interface cable to PC:	RJ11 4/4 to DSub9: 

ENVIRONMENTAL CONDITIONS	
Operating temperature	0 to +50ºC
Storage temperature	-10 to +65ºC
Relative humidity:	up to 95%

OPERATION CONTROL	*LED at the front panel:* red: instrument off green: instrument on orange: stand by

DIMENSIONS	length: 185 mm; width: 110 mm; height: 45 mm; weight: 630 g;

9.2 Analog Output and External Trigger

The device is supplied with a dual programmable 12bit analog output with galvanic isolation and an external trigger input.

- **ANALOG OUTPUT**

GENERAL SPECIFICATION - ANALOG OUTPUT

Channels	2
Connector	BNC
Resolution	12 bit
Output range	0 to 4095mV (±2mV max. error)
Galvanic isolation	500V rms
Shortcut protection	Yes
Programmable to	temperature, amplitude, phase by software

Equivalence coefficients :

temperature	1 :: 0.1	(i.e. : 208 mV = 20.8°C)
amplitude	1 :: 10	(i.e. : 2220 mV = 22200 relative units)
phase	1 :: 0.025	(i.e. : 1100 mV = 27.50°)

Update rate:
The update rate is dependent on the sampling rate of the software.
If an external trigger is used, the update rate is equivalent to the trigger pulse rate.

DC SPECIFICATION - ANALOG OUTPUT

Resolution

temperature	± 2mV ➔ ± 0.2°C
amplitude	± 2mV ➔ ± 20 relative units
phase	± 2mV ➔ ± 0.05°)

Accuracy error ± 10mV

- **EXTERNAL TRIGGER INPUT**

GENERAL SPECIFICATION - EXTERNAL TRIGGER INPUT

Channels	1
Connector	BNC
Input voltage range	TTL-compatible / up to 24V
Trigger mode	Low-High-Low (Input must be kept Low for at least 50µs)
Normal state	no current
Isolation	500V rms

Timing Specifications:

Min rise &fall time for trigger	15ns (see TTL-specification)
Max rise &fall time for trigger	2 ms
Min pulse length	3 ms
Min pause length	10 ms
Min periode length	13 ms

9.3 Technical Notes

Power Adapter

pHOptica micro should always be used with the original power adapter (110-220VAC/12VDC). As an alternative power source a battery can be used that meets the DC input voltage given in technical specification. The battery adapter cable is available as an additional accessory.

Analog Outputs

WARNING: The analog outputs are not protected against any input voltage! Any voltage applied to the analog outputs can cause irreversible damage of the circuit.

RS232 Interface

The unit uses special interface cable. Another cable can cause the unit's malfunction.

Optical Output (ST)

The ST connector is a high precision optical component. Please keep it clean and dry. Always use the rubber cap to close the output when not in use.

9.4 Operation Notes

Measurement

To achieve the highest accuracy pHOptica micro should be warmed-up for 5min before starting the measurement. Please see the details of measurement process described in pHOptica micro manual.

Temperature Compensation

No other than supplied temperature sensor could be used with the unit. The use of any other temperature sensor can damage the device.

10 Appendix

10.1 Basics in Optical Sensing

10.1.1 Major Components of Fiber-Optic Microsensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarisation). Light acts as the carrier of the information.

The major components of a typical fiber optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilised in a solid matrix)

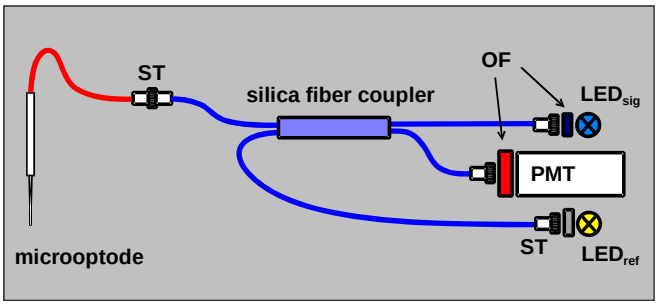


Figure 11.1 Schematic drawing of the optical setup of a measuring system with Microsensors
(LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector)

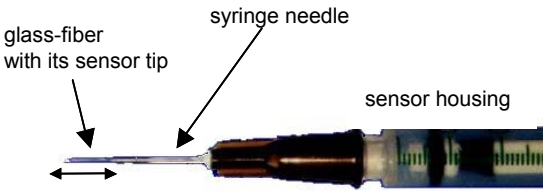


Figure 11.2 *Scheme of a sensor*

10.1.2 Luminescence Decay Time

The pHOptica micro measures the luminescence decay time of the immobilised luminophore as the analyte dependent parameter.

$$\tau = f([O_2]) \qquad (\mathbf{1})$$

The pHOptica micro uses the phase-modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is exited with a sinusoidally intensity modulated light, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exiting and emitted signal. This phase angle is shifted as a function of the analyte concentration. The relation between decay time τ and the phase angle Φ is shown by the following equation:

$$\tau = \frac{\tan\Phi}{2\pi \cdot f_{mod}} \qquad (\mathbf{2a})$$

$$\tan\Phi = 2\pi \cdot f_{mod} \cdot \tau \qquad (\mathbf{2b})$$

$$\tau \equiv \tan\Phi \equiv \Phi \equiv f([O_2]) \qquad (\mathbf{2c})$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency

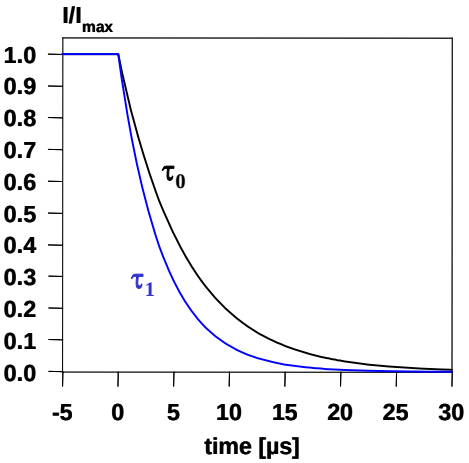


Figure 11.3 *Schematic of the single exponential decay ($t_0 > t_1$).*

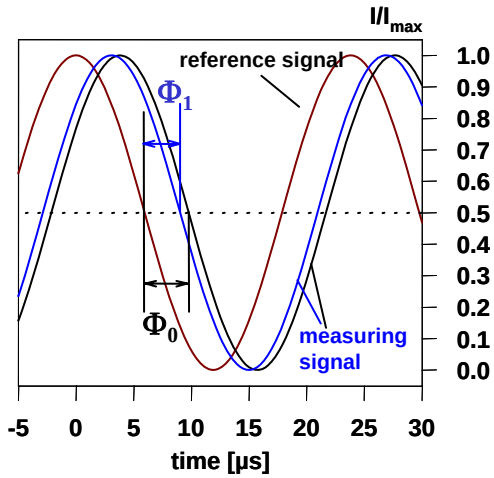


Figure 11.4 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

10.1.3 Advantages of Optical Lifetime Based Sensors

- the signal is independent of changes in flow velocity;
- they are insensible towards electrical interferences and magnetic fields;
- long-term stability and low drift;
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarisation, information such as decay time or delayed fluorescence);

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement.

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;
- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;
- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ Photobleaching and leaching of the indicator dye has no influence on the measuring signal;
- The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

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- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ Photobleaching and leaching of the indicator dye has less influence on the measuring signal;

The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

10.1.4 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

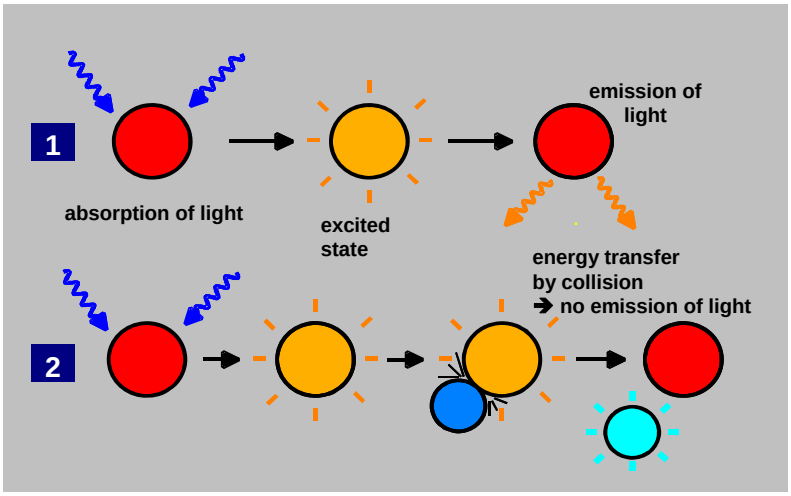

Figure 11.5 *Principle of dynamic quenching of luminescence by molecular oxygen*
(1) Luminescence process in absence of oxygen
(2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.
A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$
$$I = f([O_2])$$
$$\tau = f([O_2]) \qquad (3)$$

I: Luminescence intensity in presence of oxygen

I_0: Luminescence intensity in absence of oxygen

τ: Luminescence decay time in presence of oxygen

τ_0: Luminescence decay time in absence of oxygen

K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)

$[O_2]$: oxygen content

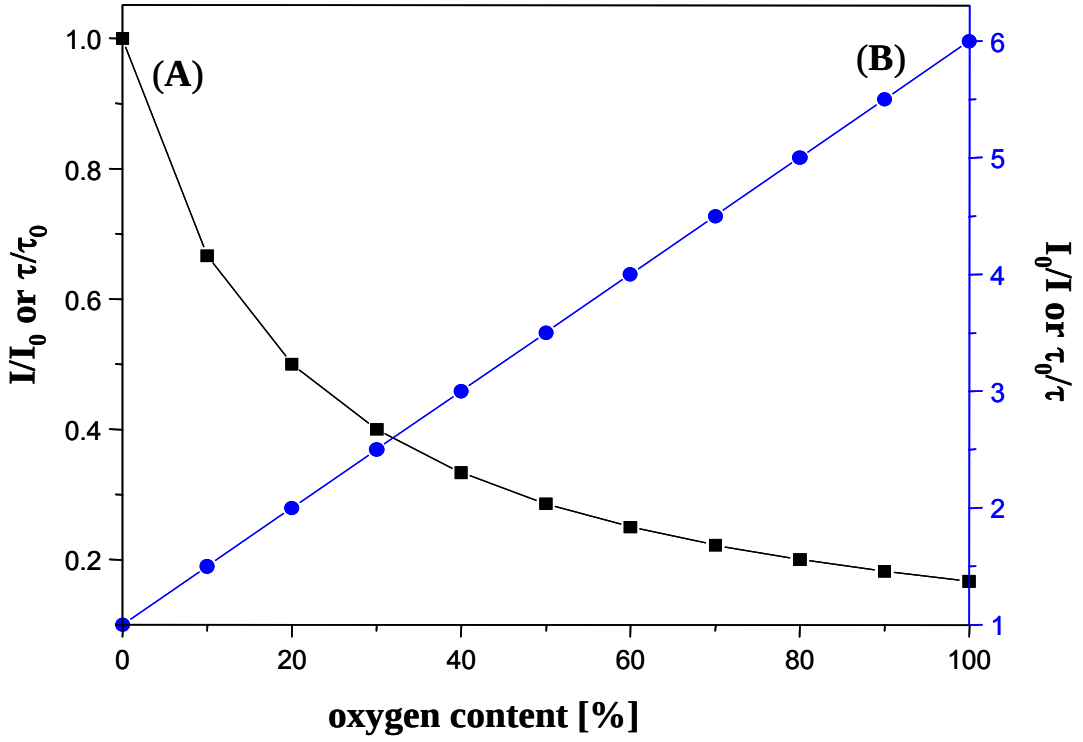

Figure. 11.6 *(**A**) Luminescence decrease in the presence of oxygen. (**B**) Stern-Volmer plot.*

Indicator dyes quenched by oxygen are, for example polycyclic aromatic hydrocarbons, transition metal complexes of Ru(II), Os(II) and Rh(II), and phosphorescent porphyrins containing Pt(II) or Pd(II) as the central atom.

10.1.5 Dual Lifetime Referenced (DLR) Optical Sensors

The measurement of intensity is simple in terms of instrumentation but its accuracy is often compromised by adverse effects such as drifts of the opto-electronic system and variations in the optical properties of the sample including fluorophore concentration, turbidity, coloration and refractive index. Therefore, efficient referencing methods are required for quantification of intensity signals. Among those, ratiometry, i.e., the measurement of the fluorescence intensity at two or more wavelengths of a single indicator fluorophore or an indicator fluorophore plus an inert fluorescence standard, is common to reference fluorescence intensity. However, this method requires two separate optical channels thus complicating the optical setup. For example, the drift in the sensitivity of both channels can be different, as

can be the intensities at two excitation wavelengths. Light scatter and signal loss caused by fiber bending (e.g. in fiber optic sensors or certain sensortiterplate readers) further contribute to effects not compensated by two-wavelength referencing.

Alternatively, the measurement of the fluorescence decay time, an intrinsically referenced parameter, is hardly affected by fluctuations of the overall fluorescence intensity. The decay time of most pH-sensitive indicator dyes, however, is in the nanosecond time scale requiring a sophisticated and expensive instrumentation which limits the use in sensor application.

WPI uses new and general logic to reference fluorescence intensity signals by decay time measurement. In contrast to the most common ratiometric method, where luminescence excitation or emission is measured at two wavelengths, this scheme uses a couple of luminophores with different decay times and similar excitation spectra. An analyte-insensitive µs-lifetime luminophore is combined with an analyte-sensitive ns-lifetime fluorophore, and a method is presented how to convert fluorescence intensity into a phase shift. Preferably, the reference dyes display decay times in the sensorsecond or millisecond time domain to simplify the opto-electronic system.

The phase-modulation (frequency-domain) method is a well-established technique for the measurement of luminescence decay times and was described in chapter 12.1.4. The phase angle Φ measured at a single modulation frequency (f_{mod}) reflects the luminescence decay time (τ) provided that the decay is single-exponential (equation 4):

$$\tau = \frac{\tan \Phi}{2\pi f_{mod}} \qquad (4)$$

In this scheme, two luminophores are used. The first, referred to as the indicator, has a short decay time (τ_{ind}), the second acting as the reference standard has a decay time in the µs range (τ_{ref}). Ideally, the two luminophores have overlapping excitation and emission spectra so that they can be excited at the same wavelength and their fluorescence can be detected using the same emission window and photodetector. The phase shift Φ_m of the overall luminescence obtained at a single frequency depends on the ratio of intensities of the reference luminophore and the indicator dye. Φ_m can be represented as the superposition of the single sine wave signals of the indicator and the reference luminophore (see Figure 11.7).

The reference luminophore gives a constant background signal (*ref*) while the fluorescence signal of the indicator (*ind*) depends on the analyte concentration. The average phase shift Φ_m directly reflects the intensity of the indicator dye and, consequently, the analyte concentration. The modulation frequency is adjusted to the decay time of the reference dye.

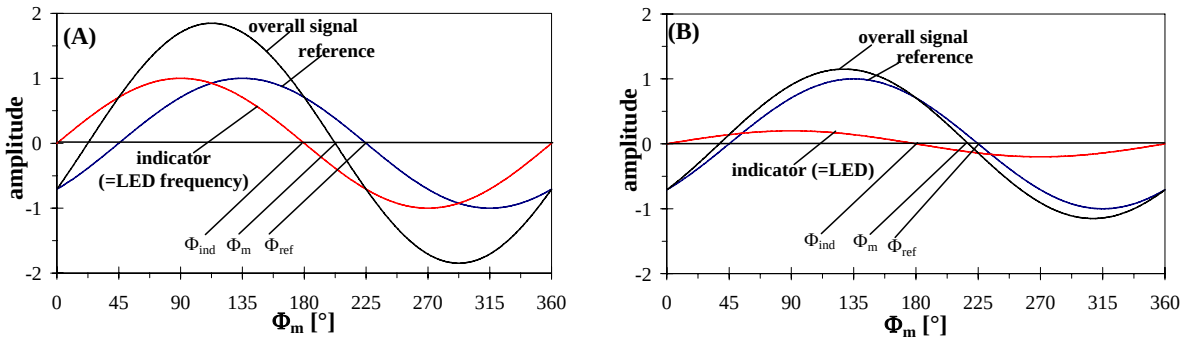

Figure 11.7 Phase shift of the overall luminescence (Φ_m), the reference (Φ_{ref}) and the indicator (Φ_{ind}). Fluorescence of the indicator in (**A**) absence and (**B**) presence of the analyte.

Equations 5 and 6 show the superposition of the phase signals of the reference dye, which possess a constant decay time and luminescence intensity, and the indicator:

$$A_m \cdot \cos\Phi_m = A_{ref} \cdot \cos\Phi_{ref} + A_{ind} \cdot \cos\Phi_{ind} \quad (\mathbf{5})$$

$$A_m \cdot \sin\Phi_m = A_{ref} \cdot \sin\Phi_{ref} + A_{ind} \cdot \sin\Phi_{ind} \quad (\mathbf{6})$$

where A is the amplitude of either overall signal (m), luminophore (ref), or indicator (ind), and Φ is the phase angle of either the overall signal (m), the luminophore (ref), or the indicator (ind), respectively. In case the modulation frequency (f_{mod}) is optimal, tan Φ_{ref} is described by equation 7

$$\tan\Phi_{ref} = 2\pi \cdot f_{mod} \cdot \tau_{ref} = 1 \quad (\mathbf{7})$$

and Φ_{ind} can be written as

$$\tan\Phi_{ind} = 2\pi \cdot f_{mod} \cdot \tau_{ind} = \frac{2\pi \cdot \tau_{ind}}{2\pi \cdot \tau_{ref}} = \frac{\tau_{ind}}{\tau_{ref}} \quad (\mathbf{8})$$

The reference luminophore has a decay time that is orders of magnitude longer than that of the indicator. Consequently, Φ_{ind} can be set equal to zero in equation 9, since at low modulation frequencies (in the kHz range) there is no phase shift.

$$\tan\Phi_{ind} = \frac{\tau_{ind}}{\tau_{ref}} \xrightarrow{\tau_{ind} << \tau_{ref}} 0 \Rightarrow \Phi_{ind} \to 0 \quad (\mathbf{9})$$

The decay time of the reference luminophore is not affected by the analyte, hence:

Φ_{ref} = constant ➔ tan Φ_{ref} = constant ➔Φ_{ref} = constant (**10**)

Therefore, equations 5 and 6 can be simplified to give 11 and 12, respectively:

$$A_m \cdot \cos\Phi_m = A_{ref} \cdot \cos\Phi_{ref} + A_{ind} \quad (\mathbf{11})$$

$$A_m \cdot \sin\Phi_m = A_{ref} \cdot \sin\Phi_{ref} \quad (\mathbf{12})$$

Dividing equation 11 by 12 results in a correlation of the phase angle (Φ_m) and the intensity ratio of the indicator dye (A_{ind}) and reference luminophore (A_{ref}) :

$$\frac{A_m \cdot \cos\Phi_m}{A_m \cdot \sin\Phi_m} = \cot\Phi_m = \frac{A_{ref} \cdot \cos\Phi_{ref} + A_{ind}}{A_{ref} \cdot \sin\Phi_{ref}} = \cot\Phi_{ref} + \frac{1}{\sin\Phi_{ref}} \cdot \frac{A_{ind}}{A_{ref}} \quad (\mathbf{13})$$

In this equation, cotΦ_m reflects the referenced intensity of the fluorescence indicator. A linear relation is obtained between cot (Φ_m) and the ratio of A_{ind}/A_{ref}, because the phase angle of Φ_{ref} of the reference luminophore was assumed to be constant. This method is referred to as Dual Lifetime Referencing (**DLR**).

10.1.6 Fluorescence (Förster) Energy Transfer (FET)

The term fluorescence energy transfer refers to a non-radiative transfer of excited state energy from a donor (D) to an acceptor (A) and results from a dipole-dipole interaction between donor and acceptor. Non-radiative energy transfer does not involve the emission and reabsorption of photons. A transfer, where the acceptor dye reabsorbs photons emitted by the donor is called radiative transfer or inner filter effect. The rate of non-radiative energy transfer (k_T) depends on the fluorescence quantum yield of the donor, the overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, and their relative orientation and distance. The theory was derived by Förster, who gave a quantitative expression of k_T between a donor and acceptor pair at a fixed separation
distance r (equation 14).

$$k_T = \frac{8.71 \cdot 10^{23} \cdot \kappa^2 \cdot \Phi_d}{r^6 \cdot n^4 \cdot \tau_d} \cdot J = \frac{1}{\tau_d} \left(\frac{R_0}{r} \right)^6 \qquad (\mathbf{14})$$

with

$$J = \int_0^\infty F_D(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 \cdot d\lambda \qquad (\mathbf{15})$$

where Φ_d and τ_d are the quantum yield and lifetime of the donor in absence of the acceptor, n is the refractive index of the medium, r is the distance between donor and acceptor and κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor. The overlap integral J, represented in equation 15, expresses the degree of spectral overlap between donor emission and the acceptor absorption. $F_d(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda+d\lambda$ with the total intensity normalized to unity and $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor at λ. The Förster distance R_0 is the donor-acceptor critical transfer distance at which radiative decay and non-radiative energy transfer are equally probable. Remarkably, the efficiency depends on the 6th power of r.

Many optical sensors exploit the principle of energy transfer. The potential of ET-based sensors relies on the fact that well-investigated absorbance based indicators can be applied by adding an analyte-insensitive fluorescent donor with a sufficiently large spectral overlap. Sensors for pH, carbon dioxide and ammonia have been realized by energy transfer from a pH-insensitive donor to a pH-sensitive acceptor. Additionally, luminescence energy transfer is a convenient way to overcome the lack of suitable lifetime based indicators since the color change of an absorber can be converted into a decay time information.

Energy transfer measurements require a constant separation distance between the D-A pair which does not vary during the excited state lifetime of the donor. This can be achieved by covalently linking donor and acceptor via spacer groups. An alternative way to fix the separation distance is the formation of donor-acceptor ion-pairs.

10.1.7 Literature

If you want to find out more about this subject, we recommend the following publications.

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pH*Optica*™

fiber-optic phase detection device

Warranty

WPI (World Precision Instruments Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from detects in material and workmanship for a period of one year* from the date of receipt. WPI´s obligation under this warranty shall be limited to repair or replacement, at WPI´s option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida,
U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air
conditioning, humidity control, or causes other than normal and ordinary usage.

To the extant that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will
not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or
indirectly by use of this product.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

Claims and Returns

• Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damage or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use
beyond the date specified on the container.

• WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all
items returned.

• Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department
for a request form.

• Goods returned for repair must be reasonably clean and free of hazardous materials.

• A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale
price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.

• Equipment which was built as a special order cannot be returned.

• Always mater In the RMA# when contacting WPI to obtain a status of your returned item.

• For any other issues regarding a claim or return, please contact the RMA department.

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