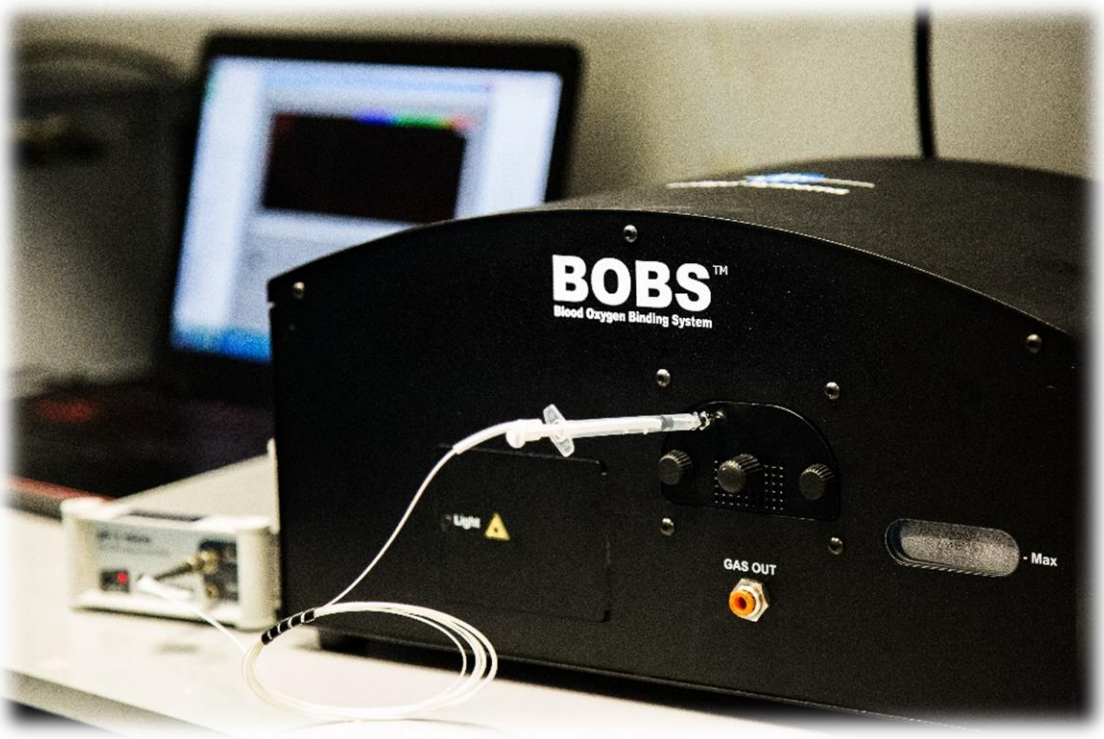
Blood Oxygen Binding System (BOBS™)

Installation and Operation Manual

Version: 1.2.1 (March, 4, 2021)



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Safety notes

Please follow these safety guidelines to protect yourself from potential hazards. Please note that this list is not exhaustive and careful handling of the device is required at all times. Loligo® Systems is not liable for any injuries arising from inappropriate operation of the device.

Please follow these general guidelines:

- **Read all instructions prior to installing, operating and servicing this product**
- **Install your equipment as specified in the instruction manual.**
- **The BOBS™ device must not be installed and operated by unqualified personnel.**
- **Connect all products to the specified electrical, gas and pressure sources.**
- **Ensure that all power cables and gas tubing are in proper condition and securely connected. Disconnect all power sources when maintenance is being performed, to prevent electrical shock and personal injury.**
- **Do not operate this instrument in excess of the specifications marked on and supplied with this product. Failure to heed this warning can result in serious personal injury and/or damage to the equipment.**
- **Thoroughly clean all surfaces that were in contact with samples.**
- **Only use the BOBS™ in well aerated spaces to avoid depletion of oxygen or harmful levels of carbon dioxide.**
- **Do not use any other chemicals or gases as specified in this manual to avoid risk to health and damage of the device.**
- **Do not use the product if it is damaged or defective.**
- **References should always be made to the Health and Safety data supplied with any chemicals used. Generally accepted laboratory procedures for safe handling of chemicals should be employed**

Symbols

Follow all **CAUTION** and **WARNING** notes marked on and supplied with this product to avoid serious injury or damage to the device.

Symbol	Meaning
	A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.
	A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.
	Danger from **electrical voltage**. Failure to head this warning may lead to serious injury or death.
	The **Biohazard symbol** denotes a potential risk to health or well-being due to the use of potentially pathogenic or infectious samples.
	**Ultraviolet light** may be present and damaging to your eyesight. Disconnect the BOBS™ device from power supply before servicing!
	Information about any **essential requirements** or actions to operate the instrument.
	**Helpful information** that may improve the usability of the instrument.
	**CE mark** indicating that the product has been assessed to meet safety and health requirements for products sold in the EEA.
	According to the directive 2002/96/EG (WEEE), any product labelled with this **WEEE symbol** must not be disposed of with the domestic waste.

Intended use of the product

The Loligo® Systems **Blood Oxygen Binding Systems (BOBS™)** is a scientific instrument and exclusively designed to measure oxygen equilibrium curves of blood pigments in research laboratories.

The use of pathogenic or hazardous blood samples is not permitted. The user must comply with standard hygiene procedures. Loligo® Systems will not be liable for any effects on health or well-being, when using hazardous samples.

Only use and apply chemicals with the BOBS™ as specified in Table 1. Loligo® Systems will not be liable for any use of chemicals and gases not contained therein.

Table 1: Use and application of chemicals approved for the BOBS™.

Chemical/Agent	Application
Blood sample	In-vitro analysis
Bromphenol Blue solution	Calibration, analysis
Deionized water	Cleaning, gas humidification
Ethanol	Cleaning
N_2 gas	Gas mixing
O_2 gas	
CO_2 gas	

The BOBS™ instrument must not be retrofitted by users.

Only original parts and accessories as well as pressure rated tubing and connectors must be used for operation and maintenance of the BOBS™ instrument.

Do not operate the BOBS™ instrument in damp and wet areas or outdoors.

The BOBS™ instrument is not designed for medical applications.

Any other use as specified in this manual is not permitted.

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1 Introduction

1.1 Product overview

Oxygen equilibrium curves of blood pigments like hemoglobin help investigate blood oxygen transport in animals and provide insightful explanations to numerous questions in biology. The **Blood Oxygen Binding Systems (BOBS™)** from Loligo® Systems ApS characterizes blood pigment oxygenation by spectrophotometric scanning of minute sample volumes combined with simultaneous pH measurement and in-built temperature control.

Features

- Flexible measurements of various blood pigment types due to highly resolved broad-range spectra (200 – 850 nm)
- Minimal sample consumption (1.5-10 µl)
- Flexible temperature setting from 5°C to 45°C
- Fully software operated and automatable experiments
- Software controlled automated gas mixing
- Simultaneous pH measurements

1.2 Components

Figure 1: BOBS™ front view.

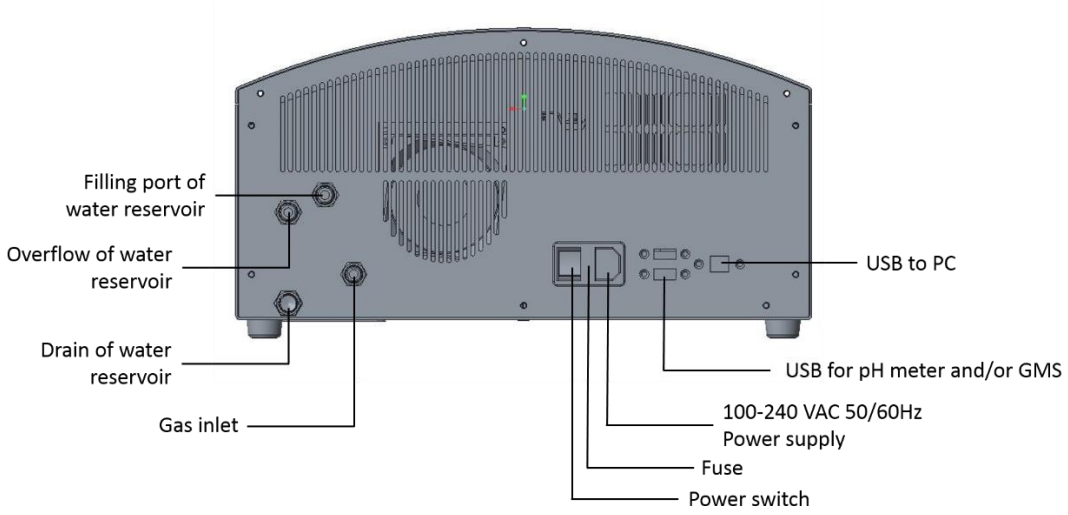


Figure 2: BOBS™ rear view.

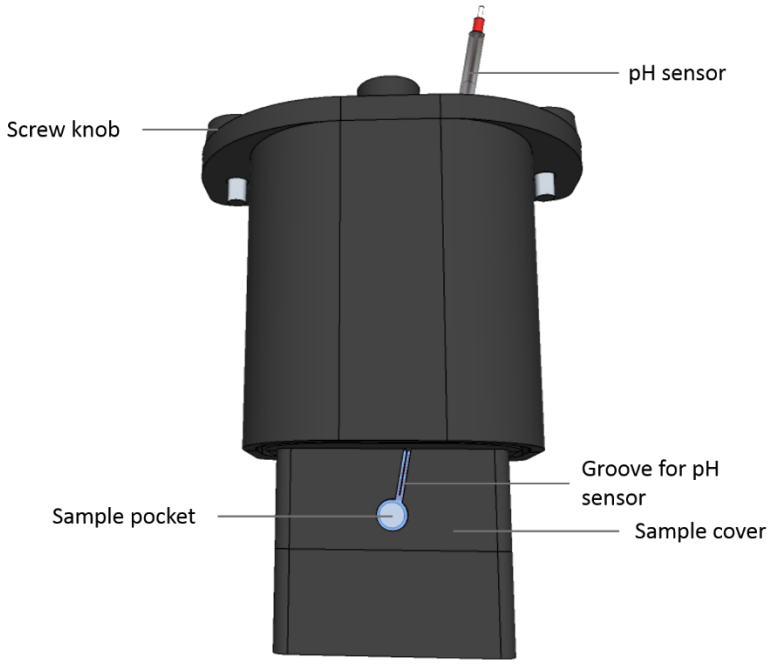


Figure 3: Sample holder

1.3 Instrument specifications

General

Specifications	Value
Sample volume	1.5 – 10 µl
Precision P_{50}*	Mean = 27.87 mmHg ± SD 1.37 mmHg / ± C.V. 1.10%
Dimensions	Height 214 mm, Width 400 mm, Depth 246 mm
Weight	~12kg
Gas connector type	6 mm push-in
Maximum input gas pressure	0.5 bar
Maximum input gas flow	500 ml/min
Communication interface	USB 2.0
Power supply	100-240 VAC 50/60Hz
Maximum current	A
Operating temperature	15-25°C (59-77°F)
Warm-up time	1-2 hours

*Measurements of P_{50} at 37°C using human blood, *n* =6, pH = 7.35

Spectrometer

Spectrometer type	Ocean Optics FLAME-S
Wavelength range	200 – 850 nm
Integration time	10 ms – 65 seconds
Optical resolution	4.06 nm (100 µm entrance slit)
Digital resolution	16 bit
Detector	Sony ILX511B CCD array (2048 pixel)
Dynamic range:	1300:1 for a single acquisition, 8.5 x 10^7 (system)
Signal-to-noise ratio	250:1 (full signal)
Dark noise	50 RMS counts
Grating	600 g/mm, best: 200-575 nm

Light source	
Type	Ocean Optics DH-mini
Light sources	Deuterium & Tungsten Halogen
Wavelength range	200 - 2000 nm
Source lifetime	1,000 hours
Stability of optical output	<0.1%
Drift of optical output	<0.2% per hour
Temperature control	
Type	Peltier controller
Adjustable temperature range	5-45°C (41-113°F)
Precision	± SD 0.01°C / ± C.V. 0.002% (*n* = 175)
Resolution	± 0.01°C
pH meter (optional)	
Type	PreSens pH-1 micro
Power supply	12 VDC/1250mA up to 18VDC/900mA
Dimensions	Height 45 mm, Width 110 mm, Depth 185 mm
Weight	630 g
pH microsensors (optional)	
pH range	5.5 – 8.5 pH
Resolution	± 0.01 pH (at pH 7)
Accuracy	± 0.05 pH (at pH 7)
Drift at pH = 7	< 0.05 pH per day (sampling interval of 1 min., may differ depending on system set-up)
Measurement temperature range	From +5 °C to +50 °C
Response time (t_{90})*	30 sec.
Compatibility	Aqueous solutions, ethanol (max. 10 % v/v)
No cross-sensitivity	Electrical fields, proteins
Cross-sensitivity	Reduced to ionic strength (salinity); a high concentration of small fluorescent molecules in

	the visible range can interfere
Sterilization procedure	Ethylene oxide (EtO), recalibration is recommended
Cleaning procedure	Water, acrylan, pepsin solution
Storage stability	24 months provided the sensor is stored in the dark

*stirred solution at 37 °C

1.4 Shipment components

Please check for completeness of the listed components when unpacking.

Figure 4: BOBS™ shipment components.

- BOBS™ instrument
- Power cable + adapter
- 2 meter 6 mm PU tubing
- Hexagonal socket screw keys
- 6 mm plug for water drain
- USB cable
- 1 x push-in hose adapter 6 mm
- Extra sample cover for sample holder
- Extra diffuser

pH meter (optional)

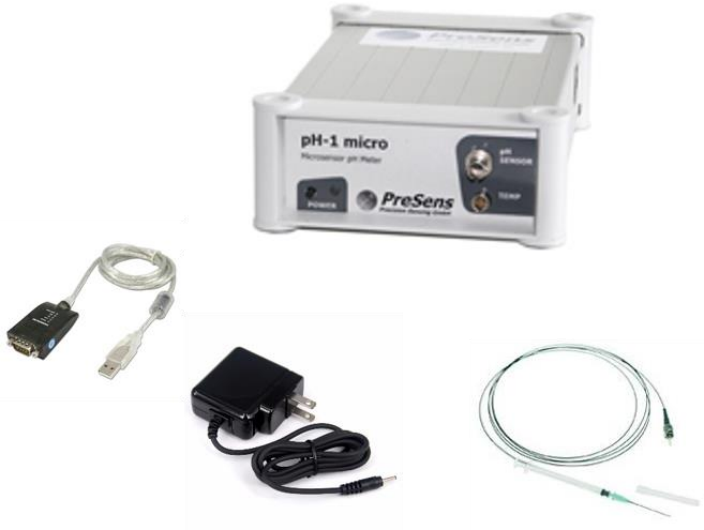


Figure 5: pH meter & accessories.

- PreSens pH-1 micro
- AC/DC power adapter
- Serial to USB converter cable
- Three customized pH microsenors

2 Installation

This chapter outlines how to perform the initial set-up of the BOBS™ software and hardware.

2.1 System requirements

- One free USB port on your PC
- Windows® 10
- PC with minimum 2.4 GHz prosessor and 8 GB RAM or better
- Recommended monitor resolution of 1280x768 pixels

2.2 Software

2.2.1 Overview

The BOBS™ software operates all hardware devices including the optional Loligo® Systems` Gas Mixing System (GMS) and the optional pH meter. Additional features such as automated experiments, in-built data processing and analysis routines provide a convenient and efficient way to obtain ready to go results.

The BOBS™ software setup installs all drivers und the user interface required to operate the BOBS™ instrument.

2.2.2 Installation

To install the BOBS™ software you need to be logged in as administrator or start the installation as administrator.

1. Turn on the computer.
2. Verify that the monitor display is set to a minimum of 1280 x 768.
3. Visit www.loligosystems.com/downloads and download the latest version of the **Blood Oxygen Binding System software.** If not logged in as administrator, right-click on `BOBS Installer.exe´ and select **`Run as administrator`** from the pop-up menu.
4. Setup now guides you through the installation procedure. Follow the on-screen instructions as they appear.
5. After installation is complete, **restart your PC**.

Communication with internal BOBS™ devices may fail if two digit COM ports were allocated by Windows®. Please change COM ports to one digit numbers in the Windows® device manager.

2.3 Hardware

2.3.1 Overview

Please follow these instructions to connect the BOBS™ and further optional devices to your PC.

2.3.2 BOBS™ installation

You must install the BOBS™ software prior to connecting the BOBS™ to the computer.

WARNING

The BOBS™ weighs 12kg. Please consult help from another person if you are unable to lift the device alone safely.

WARNING

Only install and operate the BOBS™ in well aerated spaces to avoid depletion of O_2 or harmful levels of CO_2.

1. Unpack the BOBS™ and place the device **safely** on an **even and stable surface**. Leave approximately **20 cm space** at each site to allow for **sufficient air circulation** at the ventilation slots. Keep the packaging for future transport.

WARNING

The BOBS™ device must not be connected to AC power supply while filling the reservoir as spilling of water may cause electrical shock.

The BOBS™ push-in fittings provide a safe and easy way to connect tubing. Simply push a 6 mm tube into the fitting to connect- or pull the tubing while pressing down the blue ring of the fitting towards the device, to disconnect the tubing.

2. Fill the water reservoir

    a. Assure that the lower push-in fitting at the rear labelled `DRAIN` is closed using the supplied 6 mm plug. If not, water will run out during filling.

    b. Connect 6 mm tubing to a 50 ml syringe without needle (Figure 6A).

    c. Fill the syringe with approximately **15-20 ml distilled water.**

    d. Connect the open end of the 6 mm tubing of the syringe to the push-in fitting at the rear labelled `FILL` (Figure 6A) and inject the water until

the water level reaches the `-Max` mark at the front window of the water reservoir (Figure 6B).

e. Disconnect the tubing and the syringe.

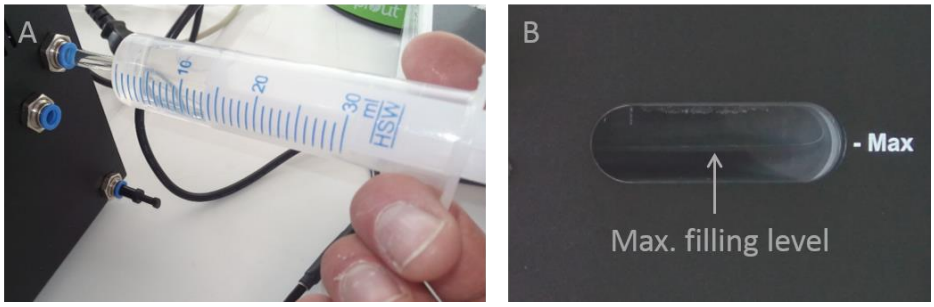


Figure 6: Filling the water reservoir.

Exceeding the recommended water level of 15-20 ml and/or gas flows of > 500 ml/min may cause spilling of water into the measurement chamber. This may block the optical pathway and/or damage the device.

3. Connect the Loligo® Systems GMS (refer to GMS Installation and Operation Manual) or an alternative gas mixing device to the orange 6 mm push-in fitting labelled `GAS IN` at the rear of the BOBS™ instrument, using 6 mm pressure rated tubing (Figure 7).

Figure 7: Rear connections

Only supply O_2, N_2 and CO_2 gas to the BOBS™ instrument. Do not exceed an input gas pressure of 0.5 bar or gas flow rates > 500 ml/min.

4. Connect the provided power cable **first** to the rear of the device and then to a **100-240 VAC 50/60 Hz** power supply.

To avoid electrical shock, use a grounded receptacle. Do not connect the BOBS™ to AC power mains without an earthed ground connection. Always connect the AC power cable to the device first and then to the power source.

Operation at AC input levels outside of the specified operating voltage range may damage the BOBS™.

5. Connect the USB cable between your PC and the USB port at the rear of the BOBS™ labelled with `PC` (Figure 7).

6. Switch on the red power button at the rear of the BOBS™ (Figure 7).

7. Start the BOBS™ software.

8. The BOBS™ is now ready to use.

Connecting other gas mixing devices

Other gas mixing systems, such as gas mixing pumps may be used with the BOBS™. In this case, the same push-in fitting labelled `**GAS IN**´ at the rear of the BOBS™ device must be used and an appropriate 6 mm tubing rated for pressures up to 2.5 bar.

Only the Loligo® Systems GMS can be connected to the rear USB port and operated via the BOBS™ software.

Do not exceed the specified maximum gas input pressure of 0.5 bar as this may damage the BOBS™ device.

2.3.3 pH meter installation (optional)

Follow these steps to connect the pH-1 micro pH meter to the BOBS™ device

1. Unpack the pH-1 micro pH meter and place the device on an even and stable surface. Keep the packaging for future transport.

2. Connect the supplied DC power adapter with the 12 VDC RJ11 socket at the rear of the pH meter and a **100-240 VAC 50/60 Hz** power supply.

3. Connect the supplied USB to serial converter cable with the RS232 socket at the rear of the pH meter and the USB socket labelled `pH´ at the rear of the BOBS™ device (Figure 7 & Figure 8A).

4. Remove the rubber cap from the optical sensor connector (ST connector) of the pH meter and keep the cap. After measurements or for storing the transmitter the rubber cap should be put back Figure 8B.

5. Remove the protective cap from the male ST plug on the optical fiber of the pH microsensor and insert it in the ST connector of the pH meter. The ST

plug has to be inserted and slightly turned clockwise to fasten it. Be careful not to snap off the optical fiber; best hold the fiber between forefinger and thumb at the bayonet lock of the male plug (Figure 8A).

Figure 8: Connect pH meter.

It is recommended to clean the ST connector and the male plug on the optical fiber with a dust free optical cleaning wipe.

6. Remove the protective needle cap while holding the pH microsensor between needle adaptor and barrel (Figure 9).

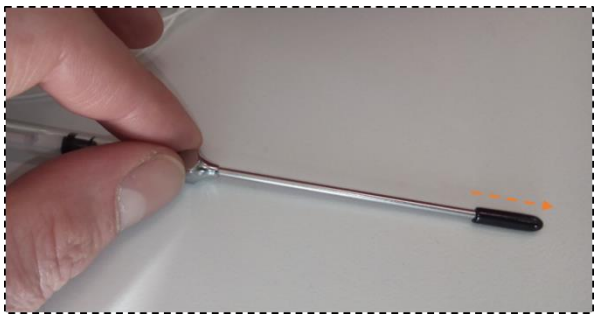


Figure 9: Remove needle cap.

CAUTION	**Always hold the pH microsensor between needle adaptor and barrel while handling, to prevent detachment of the steel needle from the barrel. Otherwise the sensor cable may break.**

7. Carefully insert the steel needle of the pH microsensor into the small 2 mm hole at the front side of the sample holder till the needle tip reaches the exit of the hole at the other side of the sample holder (Figure 10A, B).

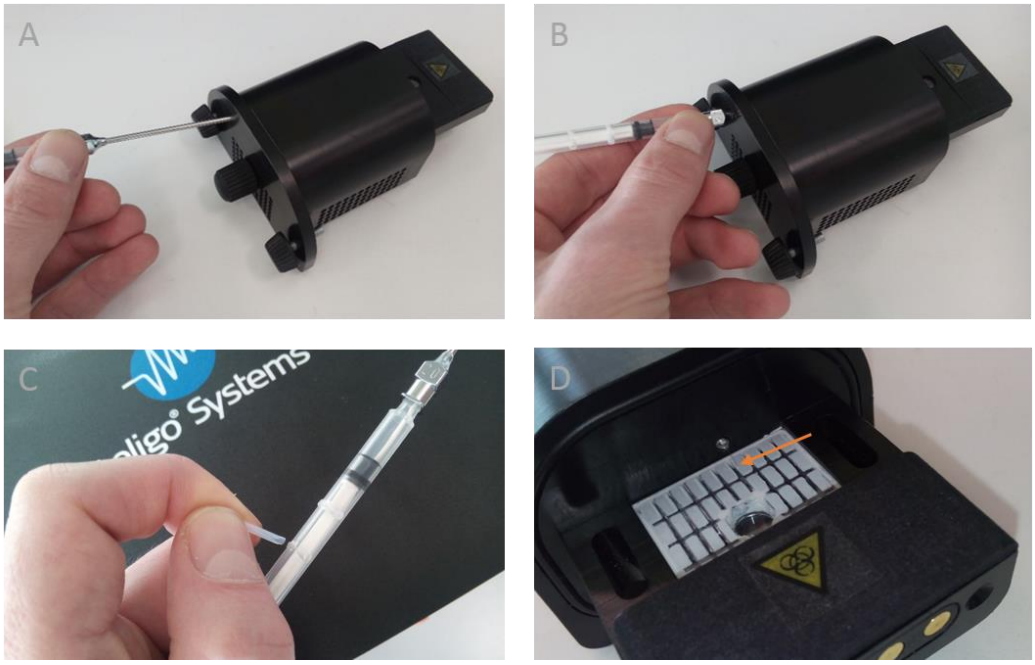


Figure 10: Insert pH microsensor.

8. Check if any particles block the exit of the hole as they could damage the sensor tip.

9. Remove the stopper at the plastic cannula of the pH microsensor to enable movement of the plunger (Figure 10C).

10. Remove the sample cover of the sample holder (Figure 10D.

11. Carefully push the plunger of the pH microsensor and observe if the sensor tip moves out in the center of the sensor groove. If not turn the pH microsensor till the sensor tip reaches a central position ((Figure 10D & Figure 11). This assures that the sensor tip moves into the sample cover groove without breaking.

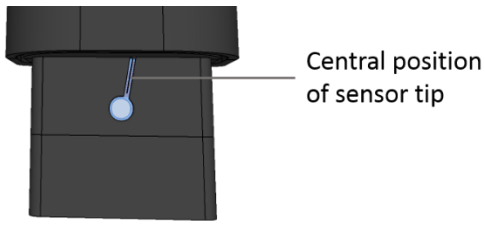


Figure 11: Sensor position.

12. Pull back the sensor tip into the protective steel needle by pulling the plunger while holding the pH microsensor between needle adaptor and barrel (Figure 9).

For more detailed information and safety instructions please refer to the official PreSens pH-1 micro user manual at www.presens.de.

3 Experiment

3.1 Overview

This chapter outlines an example experiment to familiarize with a typical workflow using the BOBS™.

3.2 Sample preparation

The BOBS™ can handle a variety of blood samples such as whole or buffered blood containing hemoglobin, haemocyanin or other blood pigment types. Due to this variety preparation of blood samples varies significantly. Therefore, we recommend consulting the respective literature for appropriate sample preparation.

3.3 Calibration

3.3.1 Spectrometer

1. Turn on the BOBS™ instrument. Make sure the sample holder is inserted and the sample glass plate is cleaned.

2. Start the BOBS™ software.

3. Go to Calibration → Spectrometer (Figure 12)

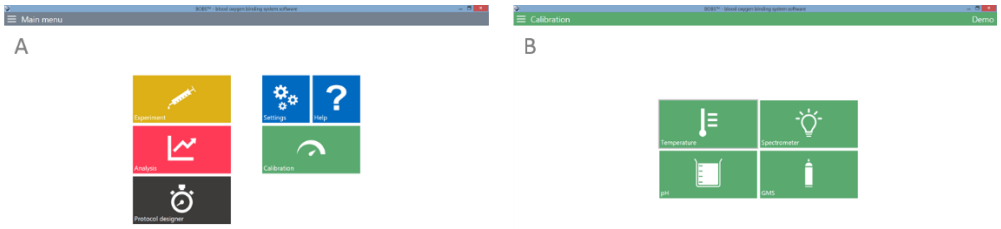


Figure 12: Spectrometer calibration menu.

4. Make sure the shutter is opened and at least one of the Halogen/Deuterium light sources is switched on (1, Figure 13).

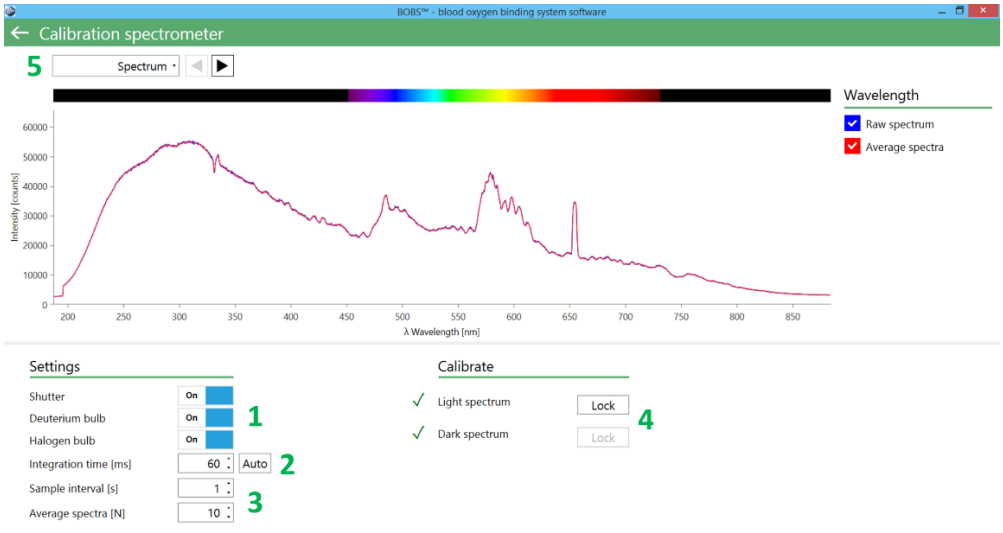


Figure 13: Spectrometer calibration

5. Check if you see a transmission spectrum as in (Figure 13, i.e. both bulbs on). If you only see a red straight line, check if the shutter and light bulbs are switched on and if nothing disturbs the light path. If you are unable to see any signal please refer to chapter 5 for troubleshooting.

6. Set the optimal integration time using the `Auto´ button (2, Figure 13). You may push the `Auto´ button several time to reach the optimal integration time, i.e. when the highest peak in the transmission spectrum reaches about 90% of the maximum intensity (~70.000 counts). You may also set the integration time yourself if you prefer to maximize the signal for a particular wavelength range.

7. Set the desired sampling interval and the number of spectra to be averaged (3, Figure 13). A setting of e.g. 10 seconds will save one complete transmission spectrum including the calculated absorbance spectrum every ten seconds. If the sampling interval is larger than the integration time excess spectra are recorded that can be averaged for signal smoothing.

8. Once the transmission spectrum is stable, push the `Lock´ button to record the light reference spectrum (4, Figure 13).

9. Close the shutter and wait again till the signal is stable. Push the `Lock´ button to record the dark reference spectrum representing any electrical noise originating at the CCD sensor of the spectrometer (4, Figure 13).

10. Select the `Calibration´ graph from the drop down menu to review both reference spectra (5, Figure 13).

11. Go to the absorbance graph. You should now see a straight red absorbance line at OD = 0 (Figure 14). If you are not satisfied with the absorbance spectrum repeat steps 8 to 11.

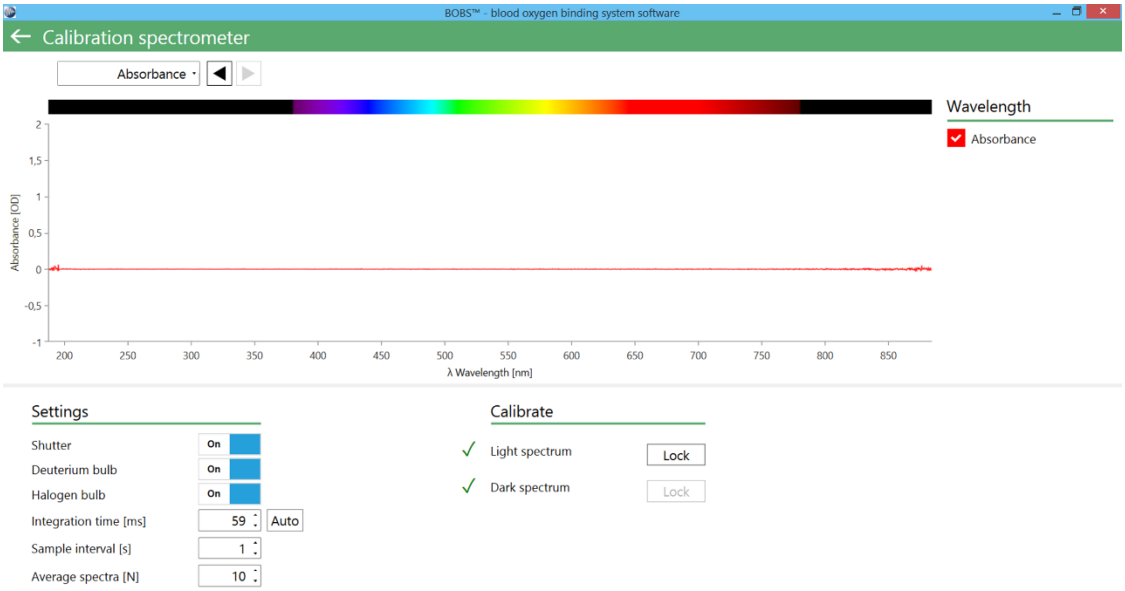


Figure 14: Absorbance spectrum.

Note any change of the integration time requires a new spectrometer calibration.

3.3.2 Temperature offset

Minute volumes of blood sample require incoming gas with a relative humidity close to 100%, to prevent desiccation or condensation. The BOBS™ instrument regulates gas humidity by modifying the temperature of the gas humidifier independently from the sample holder. To determine the optimal temperature offset between the gas humidifier and the sample holder at your experimental temperature, follow the below steps.

1. Turn on the BOBS™ instrument and allow to warm-up for about 1-2 hours
2. Go to the main menu and select ´Experiment´.
3. Set the desired experimental temperature (1, Figure 15).

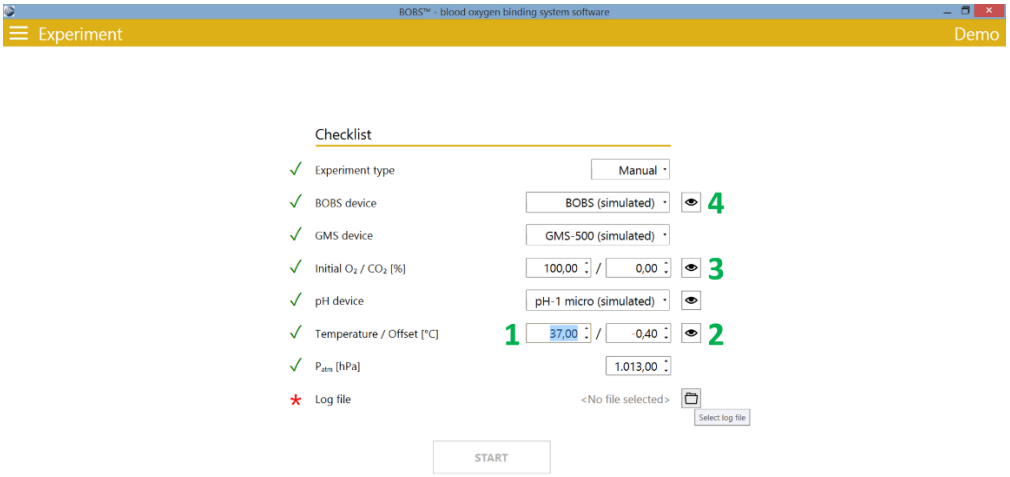


Figure 15: Temperature offset.

4. Set the temperature offset value (1, Figure 15) using Table 1 for orientation.

5. Click on the eye symbol to monitor the temperature till set values are stable (2, Figure 15).

6. Ensure gas is turned on and at full flow but NOT exceeding 500 ml/min.

7. Preview the light spectrum and observe the transmission spectrum. Then, increase the offset in minute steps (i.e. 0.1°C) till the transmission spectrum starts decreasing. At this point condensation occurs on the sample glass plate reducing light transmission. Wait for about 10 min at each step to allow the humidifier to equilibrate to the new temperature setting.

8. Reduce the offset again in smaller steps till the transmission spectrum reaches its original position (i.e. peak at 90% intensity).

Table 1: List of recommended initial offset values. Values were determined at 22°C assuring sufficient aeration at air slits and 15-20 ml filling level of the water reservoir. Offset values may differ between labs due to varying ambient conditions.

Sample temperature [°C]	Humidifier temperature [°C]	Offset [°C]
10	11,0	1,0
15	16,4	1,4
20	23,0	3,0
25	28,0	3,0
30	33,5	3,5
35	38,7	3,7
37	41,2	4,2
40	44,5	4,5
45	50,0	5,0

A consistent warm-up time for 1-2 hours at the desired experimental temperature improves the reproducibility of temperature offset values, as this allows hardware components of the BOBS™ instrument to reach thermal equilibrium.

	Changing the temperature offset in minute steps (e.g. 0.1°C) combined with sufficient equilibration time at each step (e.g. 10 min) are more efficient in finding optimal offset values, than large, short increments.

3.3.3 pH microsensor

Overview

All pH microsensors are pre-calibrated by the manufacturer under standard conditions. Calibration data are listed on the Final Inspection Protocol of the pH microsensors, which is provided together with each sensor. However, we recommend re-calibration of your pH microsensors if the ionic strength of your blood samples differs from the manufacturer`s calibration buffer (i.e. PBS 40 mmol/l, 140 mmol/l ionic strength). Furthermore, each measurement bleaches the sensor dye that may cause drift of the pH signal. We therefore recommend checking the accuracy of each pH microsensors regularly and to re-calibrate if necessary.

Multipoint calibration

A multipoint calibration (as opposed to one point calibration) yields best accuracy and is recommended for blood samples, with disturbing properties e.g. background fluorescence.

1. Obtain five or more buffers of known pH with similar ionic strength as your blood sample. The buffers should cover your target pH range (e.g. between pH 7.0 – 8.0).

Before calibration it is recommended to equilibrate the pH microsensor in the calibration buffer for a few hours or overnight.

Please do not use buffers containing dyes for calibrating pH microsensors. Buffers containing dyes, as often used for pH electrodes, can interfere with chemical optical sensors.

2. Start the BOBS™ software.

3. Go to Calibration → pH

4. Under `Settings´ set the sampling interval and the number of samples to be averaged (1, Figure 16). Larger sampling intervals will reduce bleaching and thus increase lifetime of your pH sensor.

5. Set the calibration temperature (2, Figure 16).

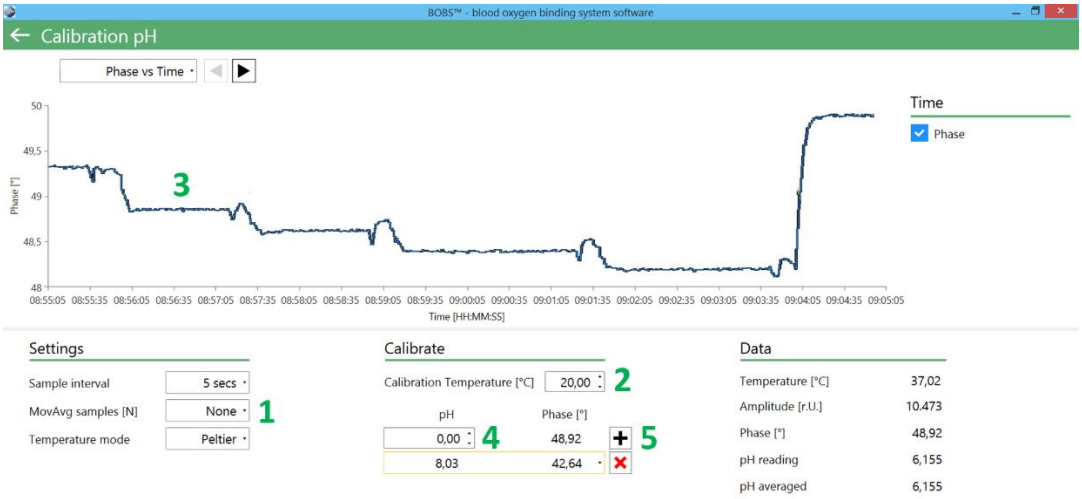


Figure 16: pH calibration

6. Pipette 3-5 µl of buffer 1 to the pH microsensor groove of the sample holder.

7. **CAREFULLY** push the plunger of the pH microsensor till the sensor tip submerges into the buffer, while **observing the sensor tip** to avoid any contact with the sample cover or the bottom of the glass plate.

The sensor tip of the pH microsensor is very fragile and must not touch any solid surfaces as this may damage the sensor tip or detach the sensor dye. Keep the sensor tip protected in the steel needle if not in use.

8. Insert the sample holder into the BOBS™ and wait until the temperature of the sample holder reaches the desired temperature.

9. Wait for about 3 minutes until the measured phase angle is stable (3, Figure 16).

10. Enter the pH value of buffer 1 (4, Figure 16) and press the + button.

11. Take out the sample holder. Pull in the pH sensor to **protect the sensor tip**! Then take off the sample cover and wipe off the buffer with an optical wipe. Use distilled water if necessary.

12. Repeat steps 6-10 with the remaining buffers.

13. Check if the fitted calibration curve crosses all calibration points (1, Figure 17).

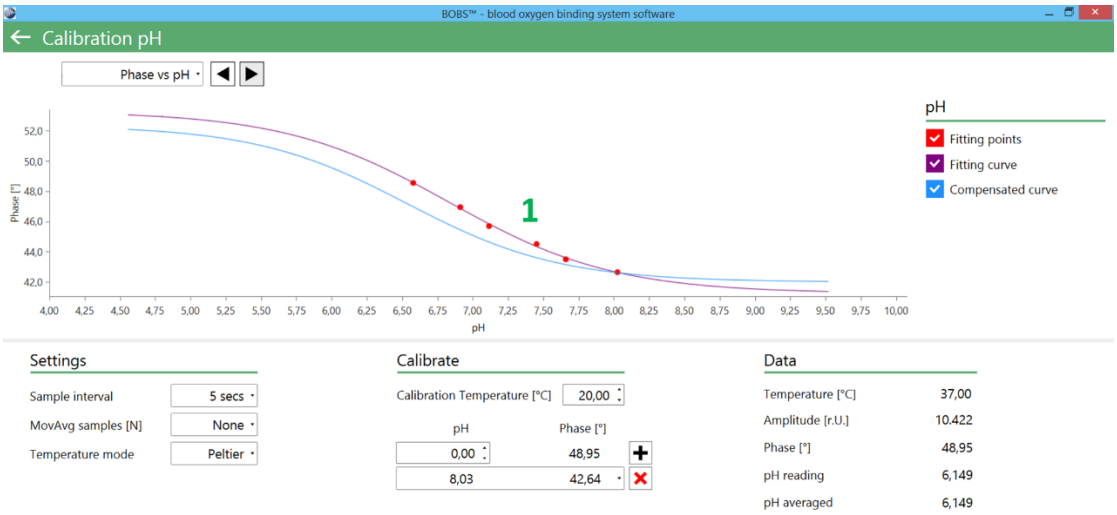


Figure 17: pH calibration curve

14. You may measure one buffer again after calibration to check if the pH is measured correctly. Otherwise repeat the calibration.

15. CAREFULLY pull the plunger to move the sensor back into the protective steel needle. Take care not to pull out the barrel from the needle adapter.

16. Proceed with sample loading (section 3.4).

The BOBS™ software has an in-built temperature compensation for pH measurements and is able to adjust pH readings for other experimental temperatures (blue calibration line, Figure 17).

Cleaning

- Clean the pH microsensor by submerging the sensor tip in a droplet of distilled water on the sample glass plate.

- When not inserted in the sample holder, clean the pH microsensor by carefully pushing the sensor tip out and letting distilled water run down the metal syringe (Figure 18).

Figure 18: Cleaning of pH sensor

We recommend to clean the pH microsensor with distilled water only. Use of other cleaning agents may require re-calibration or damage the sensor dye.

3.4 Sample loading

1. Take out the sample holder and place it on an even surface

2. Place the sample cover over the sample glass plate. For measurements without pH microsensor use the sample cover without groove.

3. Using a microtiter pipette, apply about 1-1.5 µl (or more for low-absorbent samples) sample to the middle of the sample holder. Spread out the sample to a circular droplet, using the edge of the sample cover (Figure 3) as a guide, until the complete optical path (5 mm diameter) is covered (Figure 19).

Figure 19: Sample loading

Skip steps 4-5 if not measuring pH

4. For pH measurements, use the sample cover with groove. Place a second 3-5 µl sample droplet into the middle of the groove, where the sensor tip will submerge (Figure 20A).

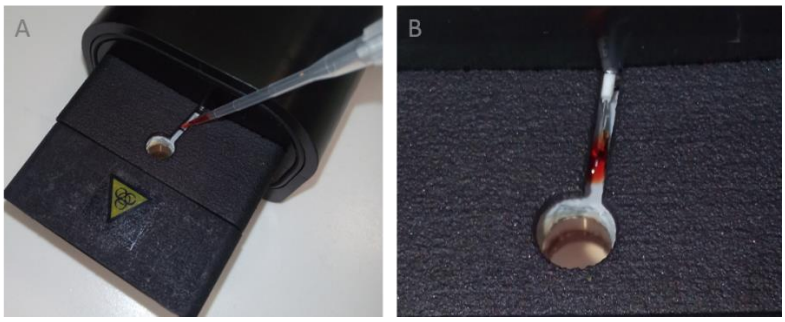


Figure 20: Sample loading pH sensor

5. CAREFULLY push the plunger of the pH microsensor and submerge the sensor tip into the blood sample (Figure 20B). Take care that the sensor tip does not touch anything else than the sample droplet. Do not position the sensor tip in the light path, where excessive light may bleach the sensor dye.

6. Move the sample holder back to the measurement unit.

Optimal sample volume depends on the optical density of the sample. Samples with high optical density such as whole blood will require lower sample volumes as opposed to purified and buffered haemoglobin solutions.

Take care that the sample droplet for the pH probe does not merge with the main sample droplet. This reduces the risk of the sample droplet flattening out and the pH probe being no longer emersed in the sample.

Applying a hydrophobic barrier around the sample droplets, using e.g. grease or a hydrophobic barrier pap pen may further help to prevent sample flattening during measurements.

3.5 Running an experiment

3.5.1 Manual

The manual mode allows changes of gas concentration during the experiment. This mode is most appropriate for establishing new protocols.

1. Choose the `MANUAL´ mode from the drop down field (1, Figure 21).

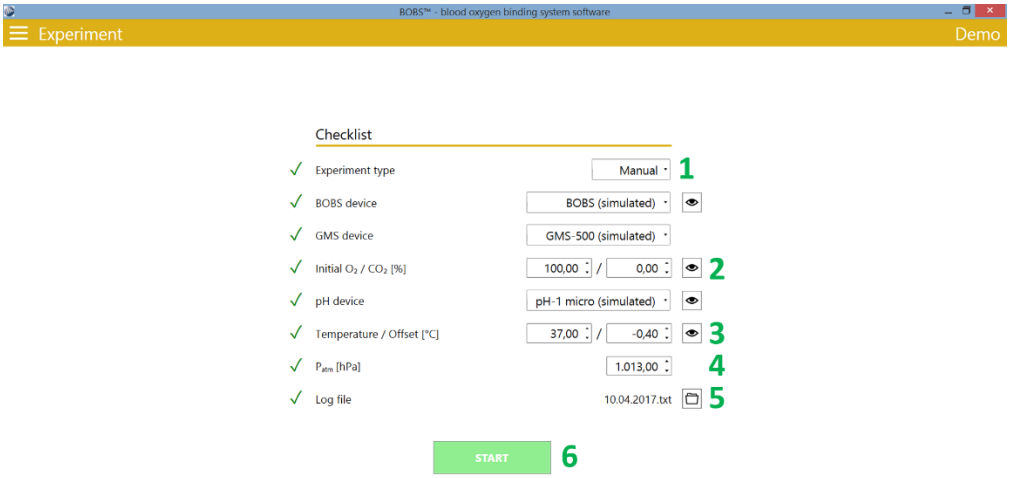


Figure 21: Manual experiment menu.

2. Set the initial gas concentrations, temperature and atmospheric pressure (2-4, Figure 21).

3. Choose the location for the log file (5, Figure 21).

4. Start the experiment (6, Figure 21).

5. Set the desired wavelength by entering the value in the corresponding text field (1) or by positioning the vertical bar in the spectrum tab (2, Figure 22).

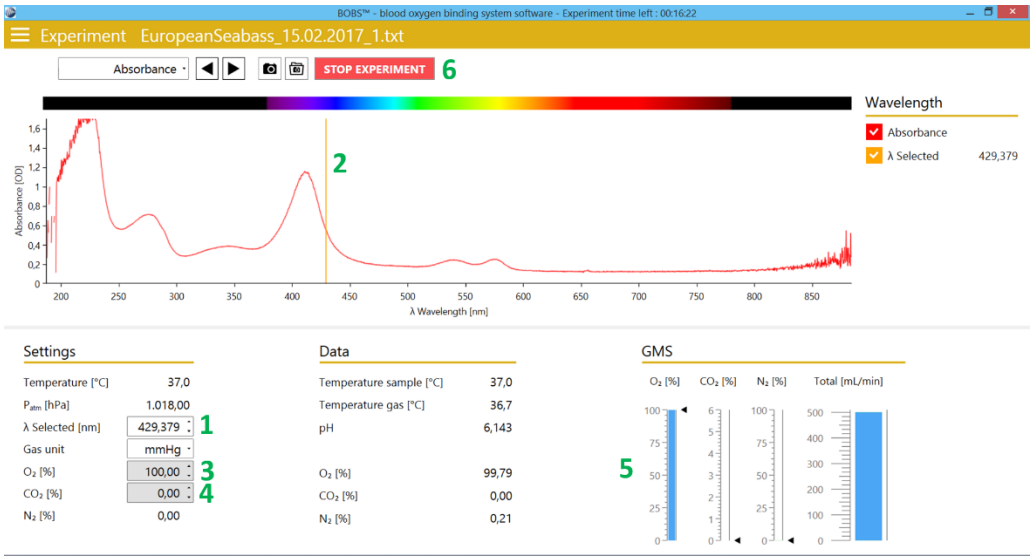


Figure 22: Manual experiment.

6. You can now freely vary the O_2 concentrations between 0.01% to 100% (3) and CO_2 between 0.03% to 6% (4). N_2 gas is adjusted automatically

and cannot be changed. Bar graphs display gas set points and current readings (5, Figure 22).

7. Stop the experiment once completed (6, Figure 22)

Please note, to exploit a larger set point range for CO_2, the total gas flow will reduce gradually at set points >3%.

The BOBS™ software saves all raw data continuously, to protect data from loss in case of unintended termination of experiments.

3.5.2 Automatic

The automatic mode runs and terminates experiments automatically, based on pre-defined protocols, without any required action by the user. This mode is most appropriate for established experiments and to generate replicated measurements.

Protocol designer

1. Go to `Protocol designer´

2. Modify the duration for the 100% and 0% O_2 calibration periods, which determines the range of pigment oxygenation (1, Figure 23).

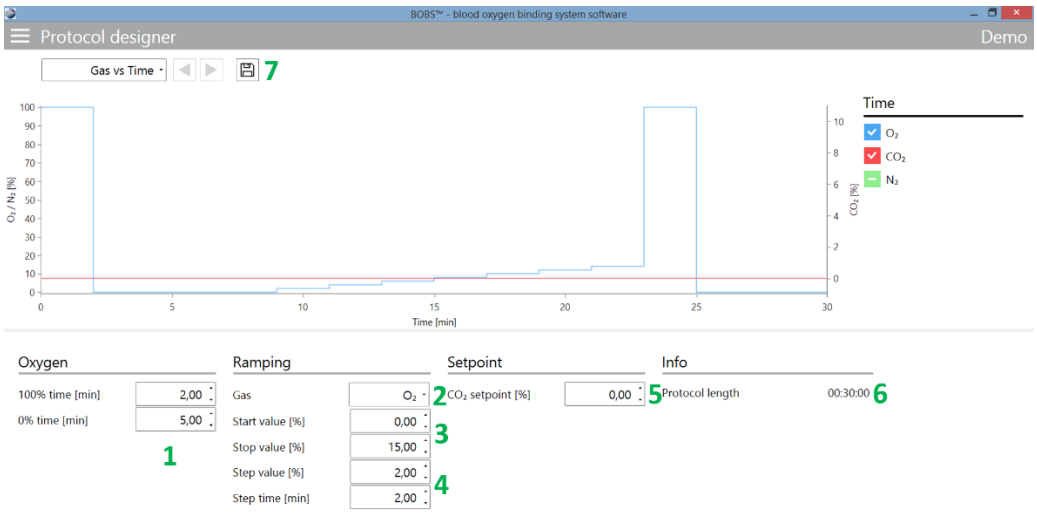


Figure 23: Protocol designer

3. Choose if O_2 or CO_2 should be ramped (2, Figure 23).

4. Choose start and stop values to determine the O_2/CO_2 ramping range (3, Figure 23).

5. Enter by how much O_2/CO_2 should increase at each step and how long each step should last (4, Figure 23).

6. Choose the CO_2/O_2 concentration, which will remain constant during ramping (5, Figure 23).

8. The duration of the total experiment is displayed (6, Figure 23). The experiment is previewed in the graph.

9. Save the protocol (7, Figure 23).

Protocols can be further customized by modifying the protocol file using any spread sheet software.

Automatic experiment

1. Go to `Experiment´

2. Load the protocol file (1, Figure 24)

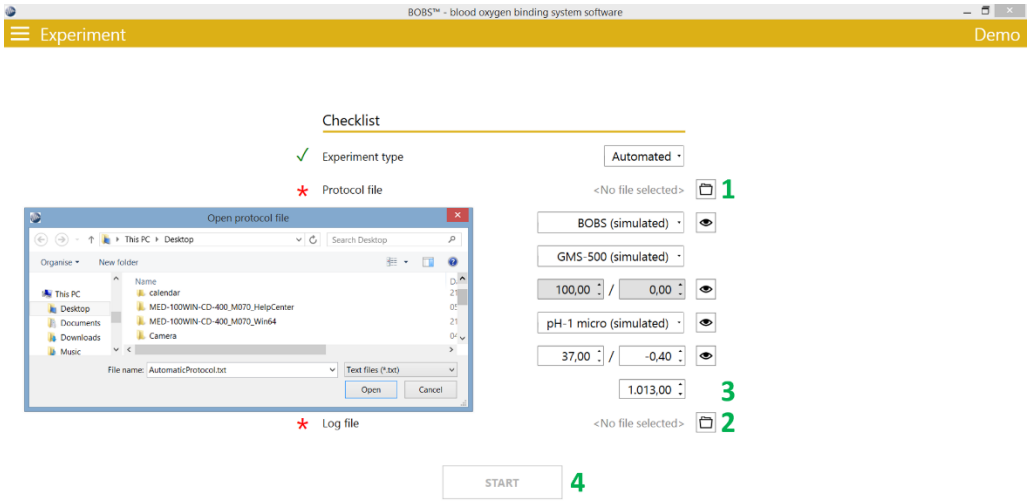


Figure 24: Automatic experiment menu

3. Load the protocol file (1, Figure 24)

4. Choose a location for your log file (2, Figure 24).

5. Set the atmospheric pressure (3, Figure 24).

6. Start the experiment (4, Figure 24).

7. Set the desired wavelength by entering the value in the corresponding text field (1) or by positioning the vertical bar in the spectrum tab (2, Figure 22).

8. The experiment will no run and terminate automatically according to the loaded protocol. Gas concentrations cannot be modified. Remaining ramping (1, visible on hover) and total time of experiment (2) are displayed (Figure 25). To abort the experiment, click on `Stop experiment´ (3, Figure 25). Click the camera symbol to take screenshots (4, Figure 25).

9. Once the experiment is completed you may review the log file or proceed to the analysis (see section 4).

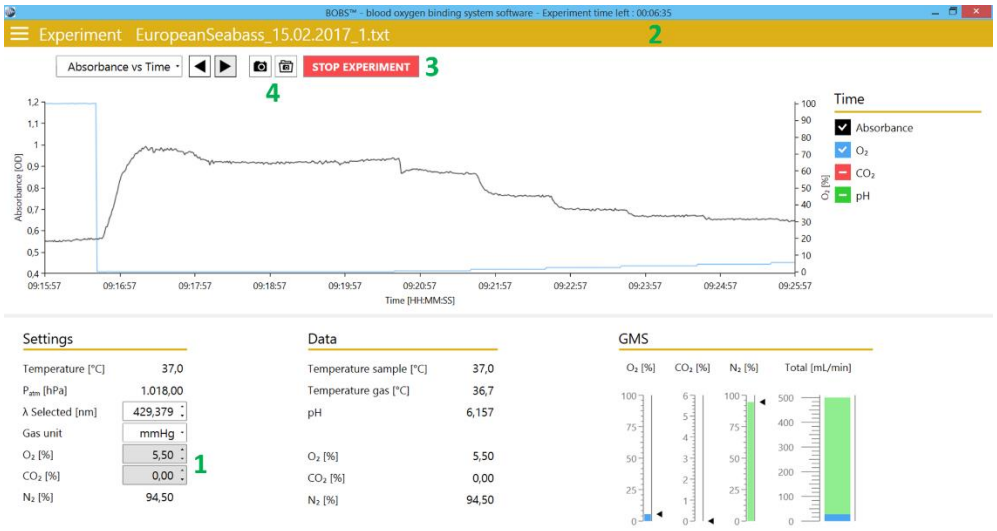


Figure 25: Automatic experiment.

Once automatic experiments complete, all gas flows are set to zero to preserve gas supplies.

3.5.3 Cleaning

1. Remove the sample holder

2. CAREFULLY move the pH sensor tip back into the protective steel needle

3. Take off the sample cover.

4. Wipe off the blood sample with an optical wipe.

5. Clean the surface with an optical wipe moistened with deionized water or ethanol solution. Make sure to remove all remains of cleaning agent to prevent interference with subsequent blood samples.

Only use deionized water or ethanol solution for cleaning. The use of other cleaning agents may damage the device or surfaces and will void the warranty.

Always comply with standard hygiene procedures and assure proper cleaning of all parts being in contact with blood sample. Contamination with infectious blood samples may cause transfer of disease. Loligo® Systems ApS will not be liable for any effects on health or well-being, when using hazardous samples.

4 Analysis

4.1 Overview

BOBS™ currently offers two modes of oxygen binding experiments:

1. pO_2-saturation experiments

   During pO_2-saturation experiments changes of blood pigment oxygenation are monitored in response to changing pO_2, while keeping pCO_2 or pH constant. This is the classical and most common type of oxygen binding experiments [e.g. 1, 2, 3].

2. pH-saturation experiments

   During pH-saturation experiments changes of blood pigment oxygenation are monitored in response to changing CO_2/pH, while keeping pO_2 constant. This approach is focusing on pH sensitivity of blood oxygenation and it is particularly useful for blood pigments with strong pH dependence [e.g. 4, 5-7].

4.2 Data Analysis

4.2.1 Loading a data file

1. Start the BOBS™ software.
2. Got to Analysis
3. Load the raw data file (1, Figure 26).
4. Select from the drop down field if your experiment was an O_2- or CO_2/pH saturation experiment (2, Figure 26).
5. Click the `START´ button to begin the analysis (3, Figure 26).

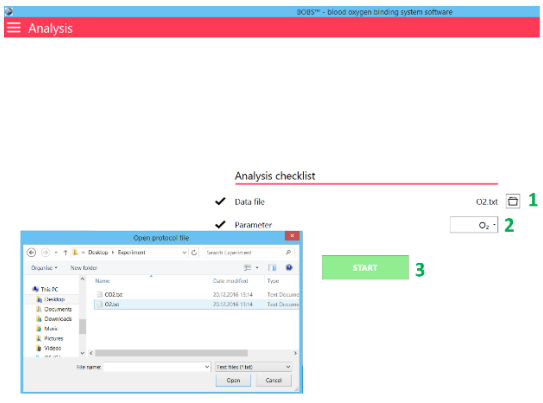


Figure 26: Load data file.

4.2.2 Analysis module

The BOBS™ analysis module comprises five main sections (Figure 27).

Figure 27: Overview analysis module.

Title bar

The title bar displays the filename and three stacked lines (sandwich menu). Click on the sandwich menu to return to the start menu.

Tool bar

The tool bar contains a drop down graph selector to choose between various graphs. The arrows or the F1 and F2 button are used for quick navigation between graphs.

The camera button creates screenshots of the current view.

The save button saves all raw- and processed data, including graphs, as a single Microsoft® Excel file.

Graph

The graph window displays various types of interactive graphs, based on the selection in the graph selector.

Graph legend

The graph legend explains the symbols and lines of the current graph display. It further displays data values that are currently selected by the vertical selection bars or closest to the mouse pointer. Selected points show a green fill color.

The display of data can activated/deactivated by clicking on the check mark adjacent to the corresponding data type.

Settings, Filters, Results

This section comprises the Settings, Filters and Results. For more details refer to section 4.3.

4.2.3 Graphs

Spectrum (multi)

In this plot the graphs representing all spectra recorded during the experiment are overlaid to provide a first general overview of your experiment (Figure 27).

Spectrum (single)

A single spectrum is plotted against time and allows review of the spectrum at any time of the experiment (Figure 28). Reference wavelength and/or the isosbestic wavelength can be selected (see section 4.3) to compute the oxygen equilibrium curve.

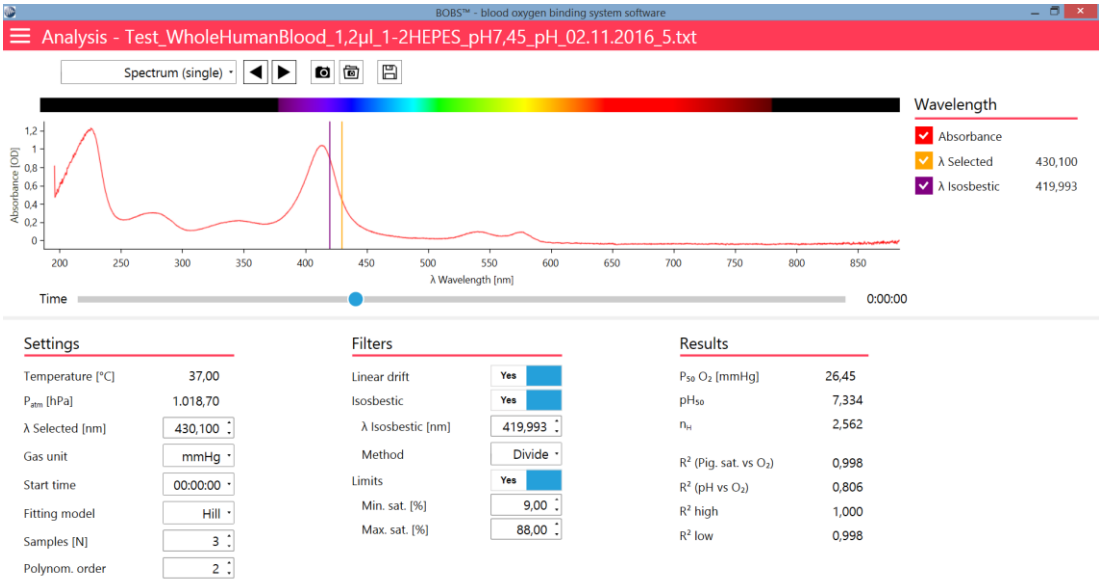


Figure 28: Graph: Spectrum (single)

Pigment saturation vs. time

This graph displays the experimental change of blood pigment oxygenation at the selected reference wavelength (Figure 29). Selected data points (black), calibration points for 100% O2 (blue) and 0% O2 (green) are displayed. By moving the yellow vertical and horizontal bars, data points can be selected (red) that will be used to create the Hill plot (Figure 29).

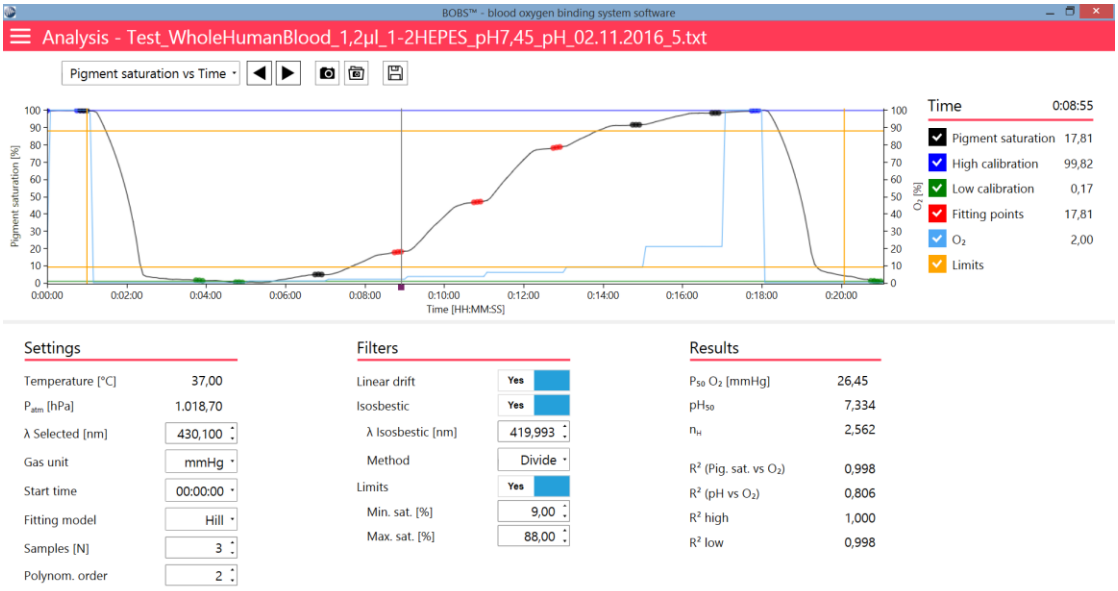


Figure 29: Graph: Pigment saturation vs. time

Hill plot

To obtain a Hill plot log(Y-/(1-Y)) is plotted versus log(pO_2), where Y denotes the fractional blood pigment oxygenation (e.g. 0.5 = 50% saturation, Figure 30). A regression line is then fitted to compute the Hill coefficient n_H, and the oxygen affinity P_{50} (see section 4.3.1 & 4.3.3). Only points selected in the `Pigment saturation vs. time´ view are used in the Hill plot.

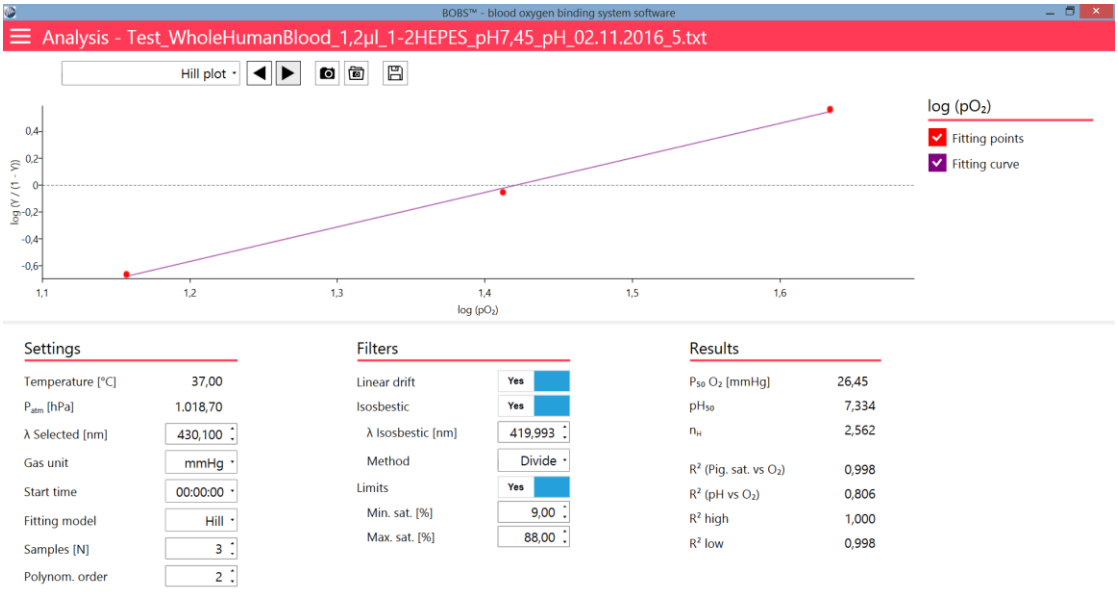


Figure 30: Data view: Hill plot

Pigment saturation vs O_2

The graph displays blood pigment saturation versus pO_2 corresponding to each ramping step (Figure 31). Based on the computed Hill coefficient a curve is fitted to the data points yielding the oxygen equilibration curve.

The yellow lines mark the P_{50} (see section 4.3.3). The red data points represent selected data points and black points all remaining data points corresponding to the ramping steps.

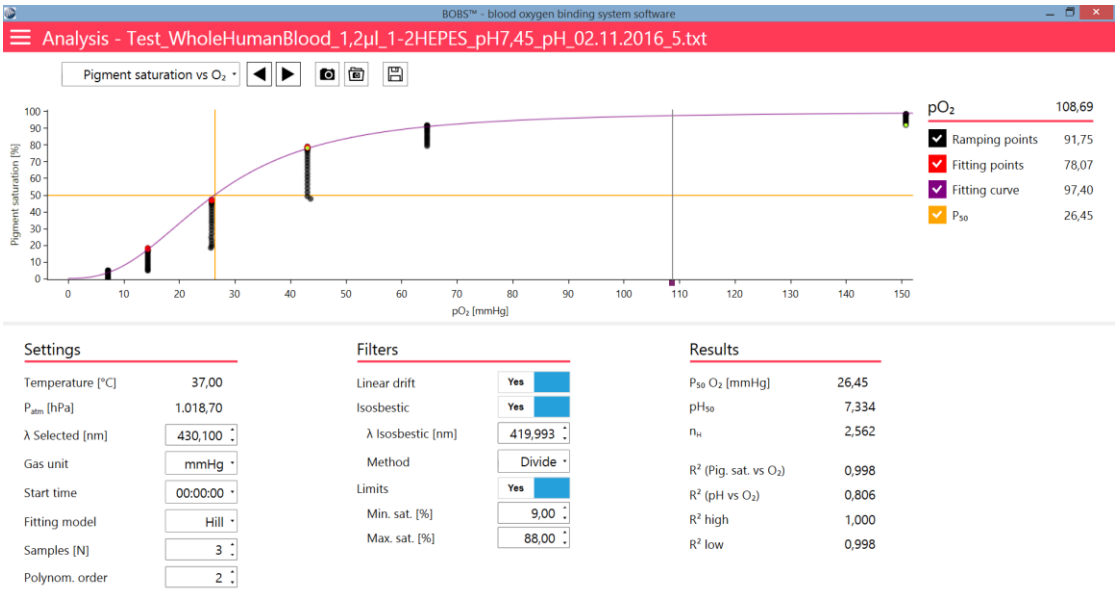


Figure 31: Graph: Pigment saturation vs O2

pH vs O_2

This graph displays the pH measured at each ramping step (Figure 32). The crossing point of the computed P_{50} (vertical yellow line) with a polynomic curve fitted to the data (purple) represents the corresponding pH indicated as pH_{50} (see section 4.3.3).

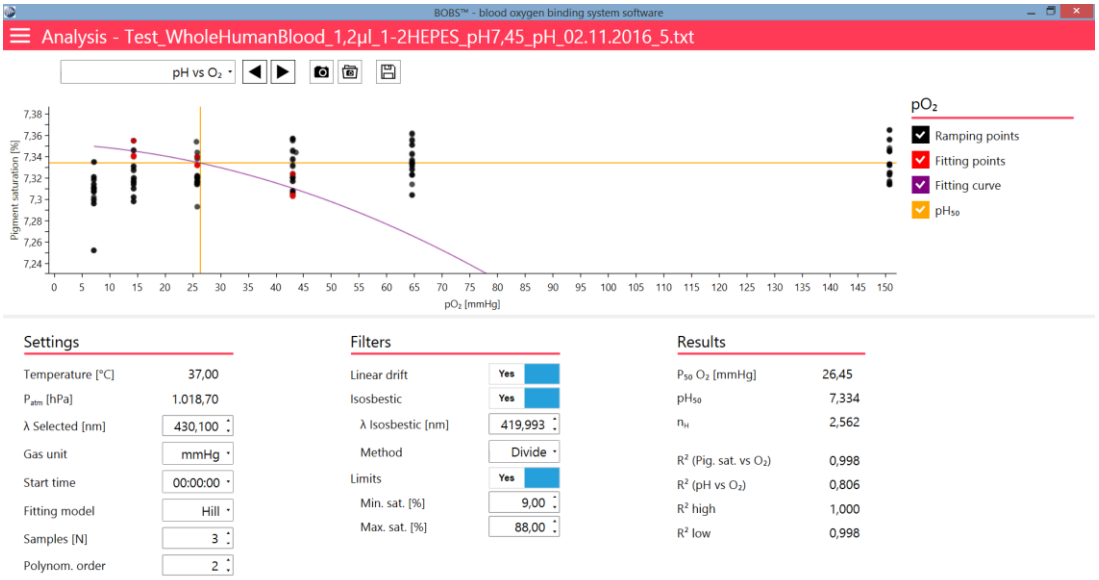


Figure 32: Graph: pH vs O_2

Absorbance vs time

In this graph absorbance at the selected wavelength is plotted versus time (Figure 33). Regression curves indicate drift of the 100% (blue) and 0% (green) calibration points from the start to the end of the experiment.

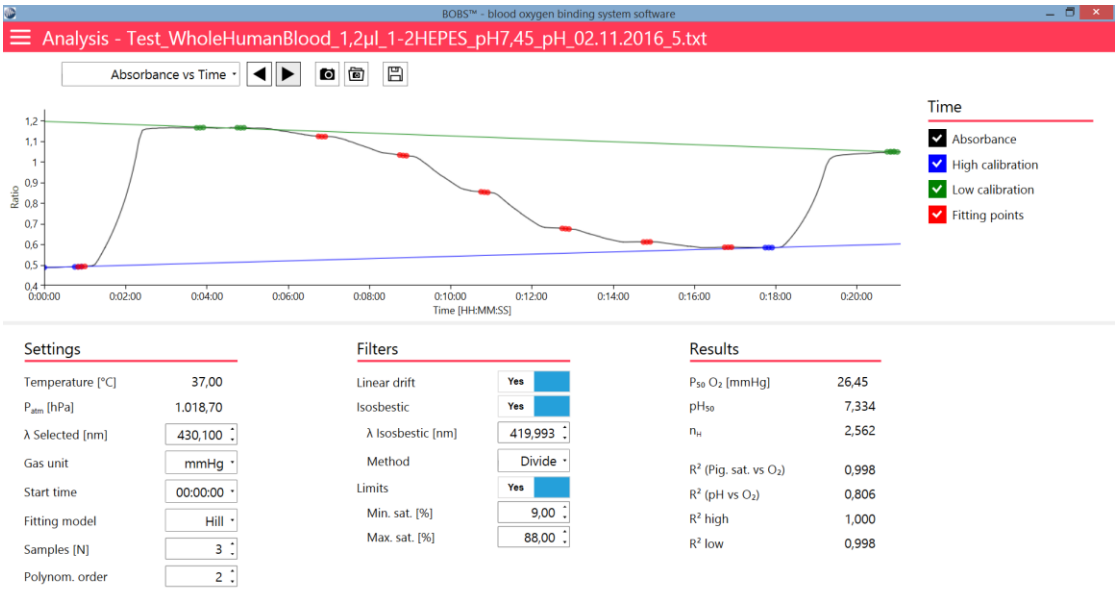


Figure 33: Graph: Absorbance vs time

Gas vs time

This graph displays the changes of set- and measured gas concentrations during the experiment (Figure 34).

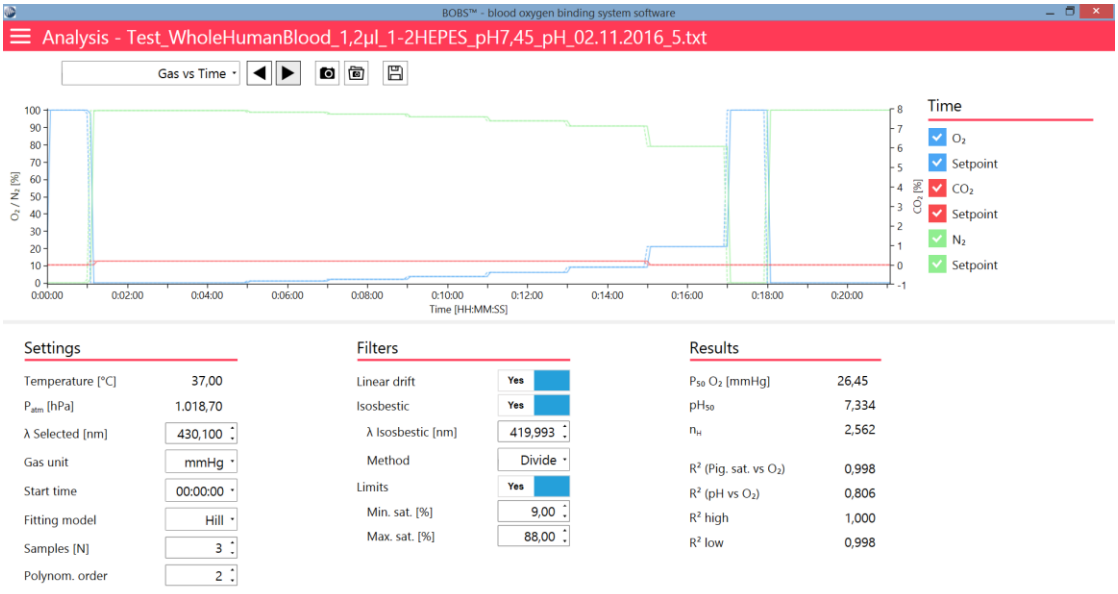


Figure 34: Graph: Gas vs time

pH vs time

This graph displays the change in pH during the experiment (requires optional pH meter, Figure 35).

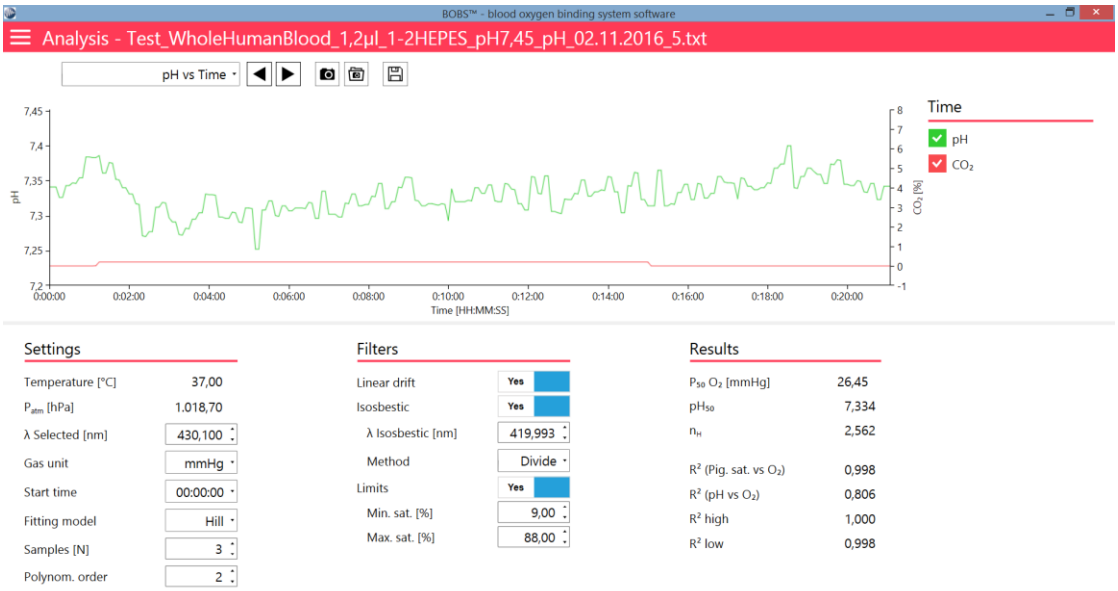


Figure 35: Graph: pH vs time

Temperature vs time

This graph displays the course of temperature during the experiment (Figure 36).

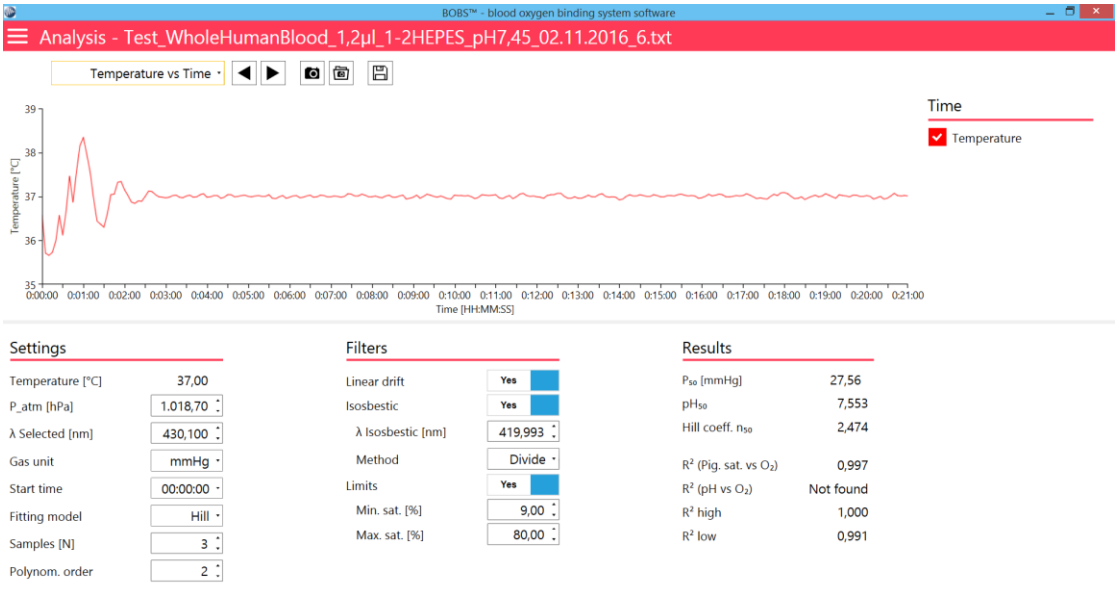


Figure 36: Graph: Temperature vs time

Isosbestic compensation (optional)

This graph displays the change of absorbance with time at the selected reference- and isosbestic wavelength, including the calculated ratio or difference (Figure 37).

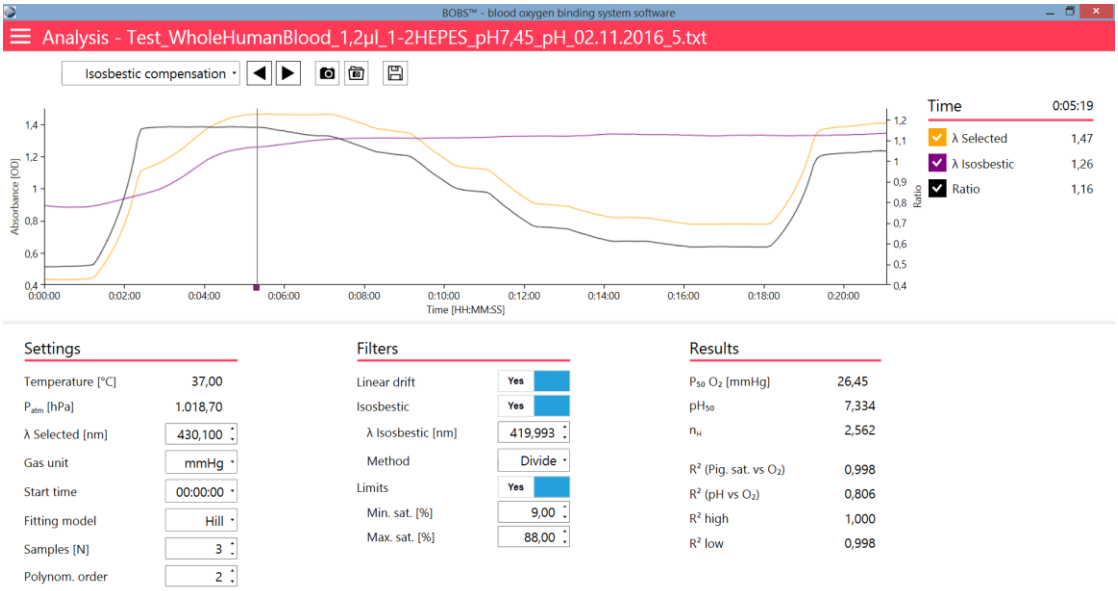


Figure 37: Graph: Isosbestic compensation

Pigment saturation vs pH (CO_2/pH experiment)

This pH-saturation diagram becomes available when analyzing a CO_2/pH experiment (Figure 38). A sigmoidal curve model (Hill or Levenberg–Marquardt, see section 4.3.1) is fitted on the data points selected from the pH ramping. The horizontal and vertical yellow lines mark the pH at half-saturation of the pigment, representing the pH_{50}.

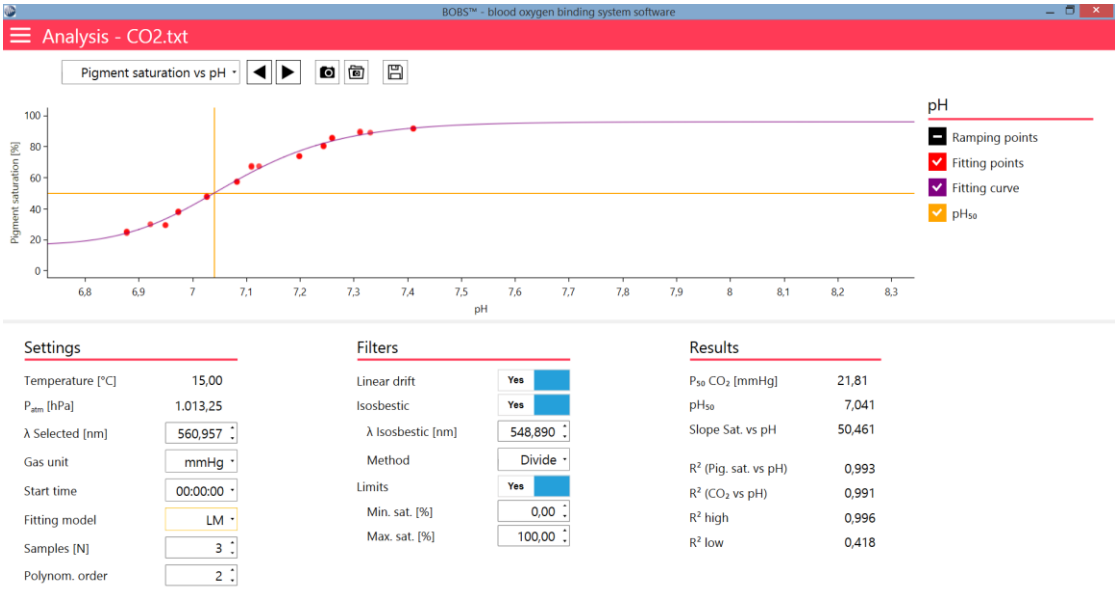


Figure 38: Graph: Pigment saturation vs pH

CO_2 vs pH (CO_2/pH experiment)

This graph illustrates the relation between the set CO_2 concentration (logarithmic scale) and the sample pH (Figure 39). A polynomic regression curve is fitted to extrapolate the CO_2 concentration at the calculated pH_{50}, denoted as $p(CO_2)_{50}$.

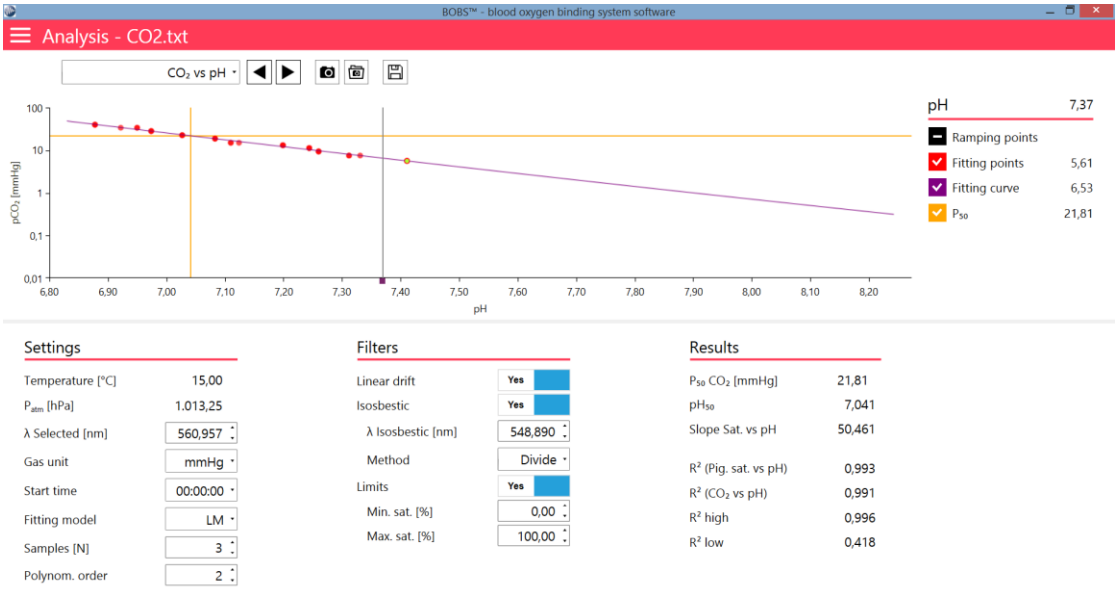


Figure 39: Graph: CO_2 vs pH

4.3 Settings, Filters, Results

The section comprises the Settings, Filters and Results. It offers options for data processing and displays analysis results (Figure 27).

4.3.1 Settings

Temperature [°C]

The temperature during the experiment

P_{atm} [hPa]

Enter the atmospheric pressure logged via a weather service or measured during the experiment.

λ Selected [nm]

The wavelength for analysis can be entered here or selected via the vertical yellow line (see section 4.2.3).

Gas unit

Choose between two gas pressure units mmHg or kPa.

Start time

Choose whether time is displayed in absolute or relative units.

Fitting model

Choose between two models to fit a sigmoidal oxygen equilibrium curve on your data according to the following fitting formulas.

Hill = Hill equation [8, 9]

$$Pigment\ saturation = \frac{pO_2^{n_H}}{(P_{50}^{n_H} + pO_2^{n_H})}$$

Levenberg–Marquardt (LM) = Five parameter log-logistic function [10]

$$Pigment\ saturation = c + \frac{(d-c)}{(1 + \exp(b(\log(x) - \log(e))))^f}$$

Samples [N]

The number of data points selected for further data processing from each ramping level, immediately before the next ramping step.

Polynom. Order

Number of polynomials applied to the fitting curve in the pH vs O_2 plot (see here).

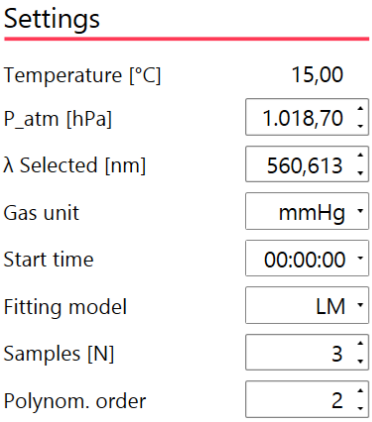


Figure 40: Settings

4.3.2 Filters

In the Filters menu several parameters may be set to assist data analysis (Figure 41).

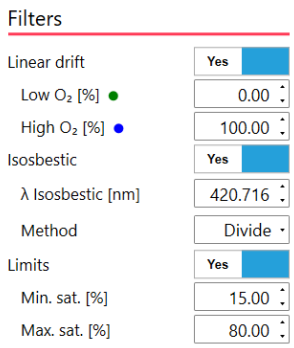


Figure 41: Filters

Linear drift

The high and/or low calibration points of the absorbance signal may change during the course of the experiment, due to autoxidation, sample desiccation or other reasons ([11], Figure 42). The `Linear drift` function in the BOBS™ software accounts for this effect by correcting the conversion to blood pigment saturation at each consecutive data point using the corresponding drift corrected calibration points. Calibration points at the beginning and end of the experiment are essential to apply the Linear drift function.

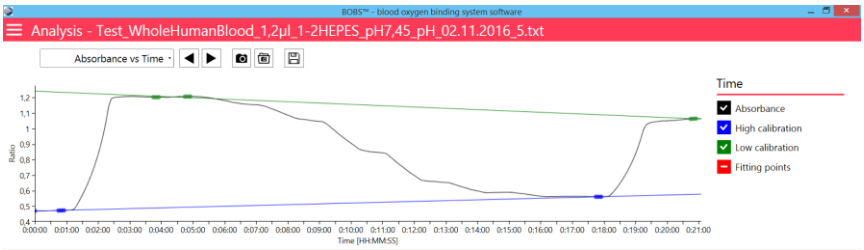


Figure 42: Linear drift

Isosbestic

Isosbestic points are specific wavelengths at which the absorbance of the sample remains constant during oxygenation changes (Figure 43). They can be used as internal reference in combination with a selected wavelength to correct for baseline drift due to e.g. light scattering or varying sample pathlengths [12, 13]. BOBS™ offers to divide or subtract the isosbestic wavelength from the selected wavelengths. The isosbestic wavelength can be set in the text field or by moving the vertical purple line (Figure 28 & Figure 41).

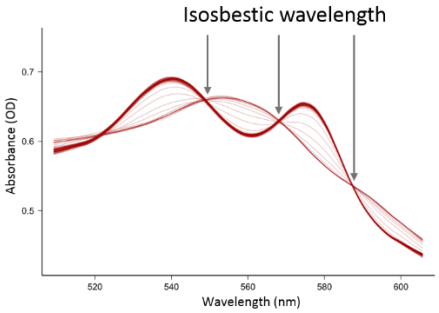


Figure 43: Isosbestic wavelengths

Limits

The Limits filter can be used to constrain the fitting of the Hill- or LM regression curve to a specific blood pigment saturation range (Figure 41 & section 4.3.3).

4.3.3 Results

The Results section summarizes the parameters calculated from the experiment (Figure 44).

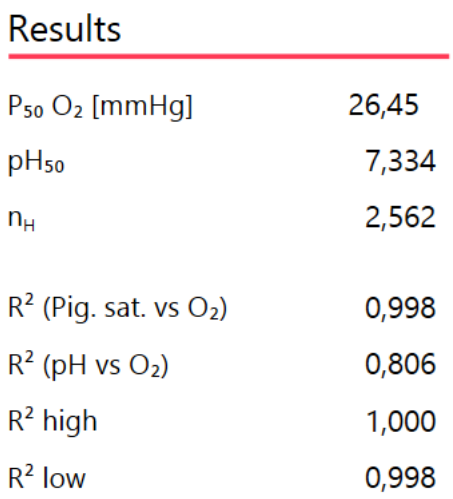


Figure 44: Results

P_{50}

The P_{50} is the oxygen partial pressure at which a blood pigment is half saturated with bound oxygen. It conventionally expresses the affinity of the blood pigment for oxygen.

pH_{50}

The pH_{50} is the blood/sample pH at which the blood pigment is half saturated with bound O_2 (see here)

Hill coefficient n_H

Blood pigments such as hemoglobin that display cooperative binding of O_2 show three distinct slopes/regions in the Hill plot (log(Y-/(1-Y)) versus log(pO_2), Figure 45). The slope at $pO_2 = P_{50}$ represents the cooperativity of the blood pigment, termed as the **Hill coefficient n_H**. The slope at high and low pO_2 equals one and represents the high affinity (R-state) or low affinity (T-state) state of the blood pigment, respectively. By selecting a specific range via the Limits filter (see section 4.3.2) one may calculate the corresponding slopes.

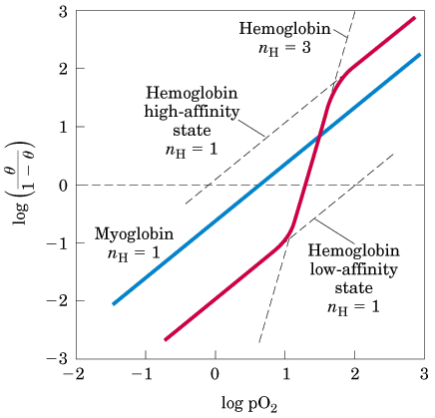


Figure 45: Hill plot of hemoglobin (Source: cbc.arizona.edu)

R²

R square denotes how well the fitting model, applied e.g. in the ` Pigment saturation vs $O_2$´ and the `pH vs O_2´plot, predicts the observed values. `$R^2$ high´ and `R^2 low´ refer to the linear regression curves of the absorbance calibration points (Figure 42). Values close to 1 indicate a good model fit.

5 Troubleshooting

This chapter gives advice and tips to solve problems that may occur while operating the BOBS™.

Symptom	Cause	Remedy
Absorbance too high	Low light transmission of sample	Dilute sample with buffer or plasma/serum Reduce sample volume Increase integration time during setup (see here)
BOBS™ software runs slow or stops unexpectedly	Insufficient RAM	Use a PC with minimum **4 GB RAM**
Curve fitting fails	Fitting model does not converge	Assure the data points correspond to a sigmoidal curve Only select data points (Sample[N]) from within each oxygen saturation level/ramp Limit the oxygen saturation range for the curve fitting to e.g. 15-80% [14]
Device communication failed	USB cable not connected	Connect the USB cable with the UBS port labelled `PC´ at the rear of BOBS™ to your computer
	Allocation of two digit COM ports by Windows	Change BOBS™ COM ports to one digit number in the Windows® device manager.
Low cooling performance	Insufficient air circulation	Provide minimum space of 20 cm before air slits to allow for sufficient air circulation
	Ambient temperature too high	Reduce the lab temperature Remove heat sources close to BOBS™

Low quality of absorbance spectrum	Absorbance too high	See above
	Light scattering by red blood cells	Increase of sample volume combined with dilution of sample with plasma/serum
	No/Low averaging of raw spectra	Increase the number of spectra to be averaged during setup (see here)
No raw spectrum	Spectrometer not recognized	Check in the device manager if the spectrometer is recognized under Ocean Optics devices
		Re-install the BOBS™ software
	Halogen or Deuterium bulbs are broken	Order a replacement bulb from Loligo® Systems
		Replace the broken bulb (see section 6.1.2)
	Fiber optic cable broken	Contact Loligo® Systems` customer service. *
No gas flow	Gas supply turned off	Turn on gas supply
	Gas tubing blocked	Assure all tubing is connected tightly to all push-in fittings and connectors
	Leaky gas connection	Check for any gas leaks in tubing or connections
Overheating alert	Sample holder exceeds maximum temperature limit	Increase temperature in smaller increments to reduce temperature overshoots.
	Reading error of the temperature sensor	Clean the electrical contacts of the sample holder with ethanol
		Apply electrical contact spray/lubricant
Sample dries out	Gas humidity to low	Increase humidifier temperature (see section 3.3.2)

		Apply a gas permeable Teflon/PTFE membrane to create a thin sample layer protected from desiccation
Sample flattens out	Low surface tension at higher measurement temperatures	Increase of sample volume if this does not compromise light transmittance Apply a hydrophobic barrier around the sample droplet using e.g. an inert grease of a hydrophobic barrier pap pen
Shift of baseline absorbance signal	Sample desiccation/condensation	Reassess temperature offset (see section 3.3.2)
	Change of sample oxygenation capacity	Intrinsic changes of sample functionality (e.g. methemoglobin formation). E.g. optimize sample preparation.
	Aggregation/movement of red blood cells during measurement	Increase of sample volume combined with dilution of sample with plasma/serum
Spectrum unstable without sample	Condensation in optical path	Always wait till temperatures reach their set point. If the sample holder temperature is far below that of the humidifier, condensation may occur on the sample holder glass.
Sudden shifts of full spectrum during measurements	Settling of spectrum after inserting sample holder	Before starting measurements, assure sufficient time for conditions to equilibrate after insertion of sample holder.
	Shutter of light source does not work properly	Go to the spectrometer calibration menu and check if the shutter blocks or transmits light when turned on and off respectively.

	Instability of power supply	Check if the lab´s power supply is stable.
Water in measurement chamber	Water spill from gas humidifier	Reduce water level in water reservoir and dry measurement chamber with optical wipe, dry compressed air or by running BOBS™ at higher temperature without sample holder. Check that incoming gas flow does not exceed 500ml/min

* Always contact Loligo® Systems ApS **prior to** shipping the instrument for service.

6 Appendix

6.1 Maintenance

6.1.1 Cleaning

- Use a moist cloth to clean the surfaces of BOBS™.
- Use optical wipes only to clean the glass plate in the sample holder.
- Only use water, ethanol, non-aggressive, non-corrosive, and non-abrasive cleaning agents for cleaning. Do not use solvents.

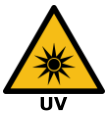
Disconnect the BOBS™ from the AC power source before cleaning. Do not wet electrical contacts. Failure to head this warning may lead to death or serious injury.

6.1.2 Replacing light bulb

The BOBS™ light source contains a Halogen and Deuterium bulb that require replacement at the end of their lifetime or if one of either the bulbs breaks. Both bulbs are contained in one housing and cannot be changed separately.

1. Disconnect the power cord at the rear of BOBS™.

Always disconnect BOBS™ from the AC power source during any maintenance work. Failure to head this warning may lead to death or serious injury.



Ultraviolet light may be present and damaging to your eyesight. Disconnect the BOBS™ device from power supply before servicing!

The light bulb may be hot and cause skin burns. Wait for at least 10 minutes to let the light bulb cool down.

2. Loosen the screw of the light source lid using the provided Hexagonal socket screw key M3 (Figure 46).
3. Remove the light source lid

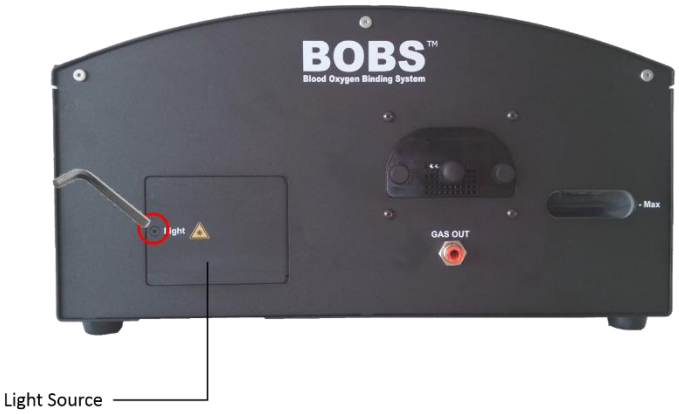


Figure 46: Light bulb replacement.

4. To remove the bulb, disconnect the cable at the rear side of the lamp first (Figure 47A). Then use the provided Hexagonal socket screw key SW 1.5 to loosen the stud screw (Figure 47B).

Figure 47: Remove light bulb.

5. CAREFULLY pull the bulb from the light source (Figure 47C).

6. Insert a new bulb. Be careful to correctly align it with the two connection holes on the PCB (Figure 47C).

7. Re-plug the cable connection on the rear side of the replacement bulb. The polarity of the connector does not matter.

8. Reattach the light source lid and secure the lid with the M3 Hexagonal socket screw.

6.1.3 Change of fuse

BOBS™ is secured with a fuse to protect the device from high currents. Follow these steps to replace the fuse if blown.

1. Disconnect the power cord at the rear of BOBS™.

Always disconnect BOBS™ from the AC power source during any maintenance work. Failure to head this warning may lead to death or serious injury.

2. Carefully lift up the fuse holder using a slotted electrical screwdriver (Figure 48A).

3. Replace the blown fuse with a new one with the exact power rating as specified on the fuse label below (Figure 48B).

4. Re-plug the fuse holder into the power socket (Figure 48C).

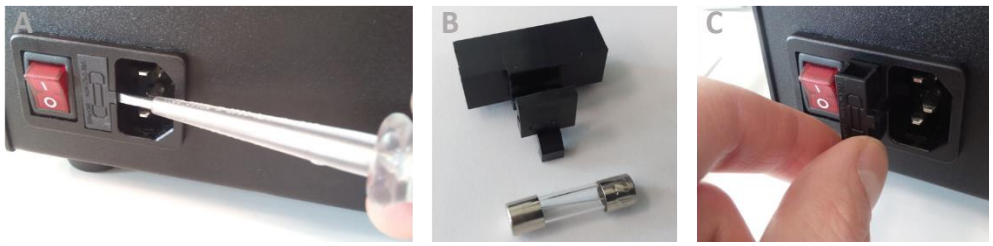


Figure 48: Replace fuse.

The use of fuses with power ratings other than specified may damage the device and will void the warranty.

6.1.4 Replace gas diffusor

The gas diffuser disperses fine gas bubbles into the gas humidifier to allow efficient humidification of incoming gas. The diffuser requires replacement at the end of its lifetime.

1. Disconnect the power cord and all other connections at the rear of BOBS™.

Always disconnect BOBS™ from the AC power source during any maintenance work. Failure to head this warning may lead to death or serious injury.

2. **Empty the water reservoir** via the DRAIN port at the rear using 6 mm PU tubing.

3. CAREFULLY turn the BOBS™ on its rear side to face the bottom towards you. Make sure the device is stable and secured from falling back.

4. Open the gas diffuser lid at the bottom using a cross tip screwdriver (Figure 49A-B).

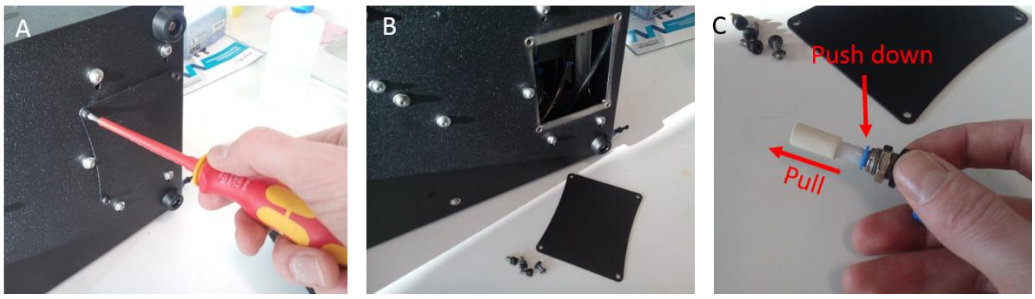


Figure 49: Change gas diffuser

5. Unplug the tubing from the push-in fitting of the gas diffuser.
6. Unscrew the L-shaped push-in fitting from the gas humidifier by turning it counter-clockwise (Figure 49C).
7. Carefully pull out the gas diffuser from the push-in fitting while pressing down the blue ring (Figure 49C).
8. Replace the old gas diffuser with the new one.
9. Return the push-in fitting back into the gas humidifier and screw it tight by turning it clockwise. Assure sealing rings are clean to allow gas tight sealing of the humidifier.
10. Reconnect the gas tubing.
11. Close the gas diffuser lid and secure it with the four screws.

6.2 Warranty

6.2.1 Warranty policy

We offer a two-year warranty against defects in material or workmanship from date of purchase. If a problem develops during that period, please contact Loligo® Systems with a detailed description. If the problem cannot be solved at a distance, we will issue you with a Return of Materials Authorization number (RMA). Loligo® Systems cannot accept responsibility for goods returned without an RMA number. Contact Loligo® Systems **prior to shipping** the product to arrange shipping, payment and documentation. Loligo® Systems will at its discretion repair or replace the instrument. The warranty specifically excludes damages caused by misuse, abuse or unauthorized modifications or repairs.

6.2.2 Limitation of Warranty

In no event shall Loligo® Systems be responsible for any damages suffered by buyer arising out of buyer's own negligence or willful acts or failure to act in connection with the storage, handling or use of Loligo® Systems Instruments by buyer or its transferee of risk of loss or damage thereto.

After the warranty period has expired Loligo® Systems offers a repair, update, and retrofit service at a fee.

6.2.3 Limitation of Remedies:

Loligo® Systems shall not be liable under any circumstances for any special, consequential, incidental, punitive or exemplary damages arising out of or in any way connected with this agreement to sell goods to buyer of the goods, including, but not limited to, damages for lost profits, loss of use, lost data, or for any damages or sums paid by buyer to third parties, even if Loligo® Systems has been advised of the possibility of such damages. The foregoing limitation of liability shall apply whether the claim is based upon principles of contract, warranty, negligence, or other tort, breach of any statutory duty, principles of indemnity or contribution, the failure of any limited or exclusive remedy to achieve its essential purpose, or otherwise.

6.3 Disposal

Before disposal, the device must be decontaminated and cleaned to protect people, the environment and property.

Always observe the statutory requirements when disposing the product.

According to the directive 2002/96/EG (WEEE), any devices supplied after August 13, 2005, to the business-to-business area this product is assigned to, must not be disposed of with the domestic waste.

Because disposal regulations may differ from one country to another, please contact your supplier if necessary.

This symbol of the crossed-out garbage bin points out that the unit must not be disposed of with the domestic waste.

6.4 References

6.4.1 Patent

BOBS™ is protected and licensed by Loligo® Systems under the following international patent.

Oellermann, M., Mark, F. C. and Dunker, E. Diffusion chamber for ascertaining different parameters of an aqueous substance. International Patent no. WO2015000453, January 8, 2015.

6.4.2 References

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6.5 Declaration of Conformity

Declaration of conformity
Konformitätserklärung
Déclaration de conformité
Declaración de homologación

The undersigned, representing the following manufacturer:

Loligo® Systems ApS
Toldboden 3, 2nd floor
8800 Viborg
Denmark

herewith declares that the product:

#BG10000 - Blood Oxygen Binding System (BOBS™)

is in conformity with the provisions of the following EU directives, including the latest amendments:

2014/30/EU EMC Directive

and that the following standards have been applied:

EN 16010-1: 2011	Safety requirements for electrical equipment for measurement control and laboratory use. Part 1: General requirements.
EN ISO 12100:2011-03	Specifications for basic terminology, principles and a methodology for achieving safety in the design of machinery.
EN 61326-1:2013	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements.
EN 61000-3-2:2006+A1:2009+A2:2009	Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions.
EN 61000-3-3:2013	Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems.

Standards for Canada and USA

FCC Part 15 Subclause B, UL 61010-1:2012 (3rd Edition), CAN/CSA-C22.2 No. 61010-1:2012 (3rd Edition)

Jannik Herskin, CEO

January, 5, 2017, DK-8800 Viborg

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