DOTS
a platform by sbi

MPS
User Guide

A handbook for multiparameter monitoring in shake flasks

sbi SCIENTIFIC BIOPROCESSING


DOTS
a platform by sbi

Table of contents

Introduction

Welcome to the MPS user guide and congratulations on choosing the Multiparameter Sensor (MPS) system to monitor your culture. This user guide will give you access to all information required to install, integrate, and use the system within your laboratory everyday life. It is strongly recommended to read this user guide prior to any installation or operation of the system.

Important aspects, hints or summaries are highlighted by grey boxes.

Important aspect:

This kind of grey box will be used throughout the complete document for the indication of important aspects, hints, or summaries.

Caveats are indicated by yellow warning signs

Dangers and risks are indicated by red danger signs

To ensure that this user guide provides you all information you need during your work with the multiparameter system, we at sbi are reliant on your feedback. Do not hesitate to contact us to share your ideas regarding errors, missing information, or incomprehensibilities so that we can improve this document and keep it up to date with your requirements.

This user guide, the MPS, the Shake Flask Adapters, and the DO Sensor Pills, may be subject to changes and improvements without further notice.

You may request the latest manual from our team of Application Scientists.

insights@scientificbio.com

In case of any questions that may arise during your work with the Multiparameter Sensors, do not hesitate to contact us.

MPS user guide revisions:

Revision 0	05.04.2024	Initial document
Revision 1	12.12.2024	▪ Added information for applications in mammalian cell culture ▪ Updated and added specifications and operating conditions ▪ Added warnings ▪ Added firmware update guide ▪ Updated general descriptions, figures, application hints and others.

Multiparameter Sensing – Overview

The MPS (Multiparameter Sensor) is an analytical laboratory device for optical online monitoring of multiple parameters in shake flasks. Parameters of common interest for microbial and cell cultures are covered and expanded continuously as part of the growing SBI DOTS Platform. The first version of MPS comes with modules that monitor cell density (biomass) as well as fluorescence at various wavelengths, and DO (dissolved oxygen), all through one single device. Typical shaker environment data, such as temperature, shaking speed (rpm) or ambient pressure, are also logged by the MPS. For DO monitoring, the MPS is combined with a novel DO Sensor Pill technology. The Sensor Pill for Dissolved Oxygen (DO) is covered with a luminescent, oxygen-sensitive dye. While the Pill circulates with the liquid inside the shake flask, its DO-signal is read out by the MPS underneath the shake flask.

Figure 1: Multiparameter Sensors monitoring biomass, fluorescence, and DO via DO Sensor Pills in a cell culture application.

The Multiparameter Sensor system consists of the MPS (Multiparameter Sensor), Shake Flask Adapter, the MPS Hub, and the DOTS Software. Sterile DO Sensor Pills are dropped into the shake flask for DO monitoring. Two types of Pills are available to meet the demands of microbial and cell culture

applications. The DOTS Software provides a customizable environment for smart monitoring of all available parameters including screening options.

The Multiparameter Sensor system can be combined with the LIS feeding system available on the DOTS Platform. Both systems act together to bring the shake flask one step closer to a smart and controlled bioreactor environment. The biomass-based feeding and the DO-based feeding applications on the DOTS Software synchronize MPS, DO Sensor Pill, and the LIS feeding systems. The core of these applications are a trigger-based smart feeding function and a PID-controller. Both can also be customized to expand intelligent feeding applications to other control parameters.

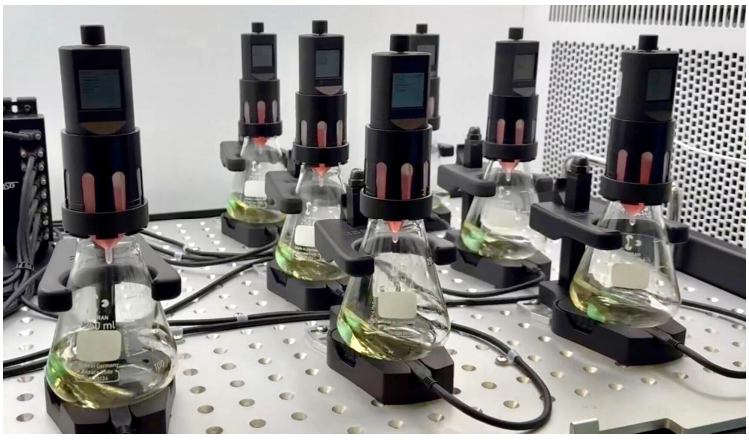


Figure 2: The Multiparameter Sensor combined with the LIS Feeding system. The online parameters biomass, fluorescence, and DO can be utilized to trigger and control feeding.

Multiparameter Sensor (MPS)

Mounted below the shake flask, the MPS combines backscatter, spectrometer, DO, and ambient sensors into one single device. Using the DOTS Software, multiple measurements can be configured, which will then be performed sequentially by the MPS. Data is collected under continuous shaking conditions and transferred to the DOTS Software via a USB-C cable. The MPS includes a

rechargeable Lithium-Polymer battery as well as other hardware components for future compatibility with wireless applications.

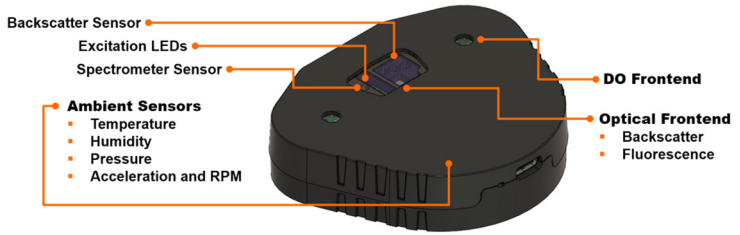


Figure 3: The MPS combines backscatter, spectrometer, DO and ambient sensors into one single device.

Sensor Pill – Dissolved Oxygen (DO)

A Sensor Pill - DO is coated with a luminescent dye capable of detecting changes in dissolved oxygen. The Sensor Pill is dropped into the flask where it circulates with the liquid. A pill identification algorithm allows the MPS, located below the shake flask, to take measurements during shaking.

Figure 4: The Sensor Pills - DO come individually sterile packaged and are added directly into the shake flask.

Sensor Pills are delivered sterile, individually packaged, and are intended for single-use in microbial and mammalian cell cultures.

DOTS Software

The DOTS Software allows users to setup experiments, visualize data, and control any connected sensors. Various types of sensors can be combined for smart applications like DO-based feeding with MPS and LIS. Instructions for the DOTS Software are not included in this manual, but are provided in the DOTS Software User Guide.

Technical Specifications

MPS and Shake Flask Adapters

Housing material	ABS and aluminum
Battery capacity (typical)	650 mAh
Power supply (via USB)[1]	
Input voltage (max. range)	4.5 – 5.5 VDC
Input current (max.)	0.5 A
Connection to PC	via the MPS USB-C connector: ▪ directly using a USB 2.0 (or higher) compatible USB-C-to-USB-A cable or USB-C-to-USB-C cable ▪ using a MPS Hub via a USB 3.0 (or higher) compatible USB-C-to-USB-A cable or USB-C-to-USB-C cable (cables are provided with the MPS Hub)
Operating and storage temperature	4 – 45 °C
Operating and storage humidity	≤ 85 % (non-condensing)
Optimal storage conditions	Dry, dark, and at room temperature
Internal temperature sensors	
Accuracy[2]	0.5 °C
Resolution	0.1 °C
Range	4 - 45°C
Internal rpm sensor	
Accuracy[3]	5 rpm
Resolution	< 1 rpm

Range	Optimal[4]: 50 - 350 rpm Functional: 0 - 350 rpm

1 The USB-C port is protected against overvoltage up to 25V and transient electric discharge surges up to ±15kV (contact and air according to IEC 61000−4−2).

2 Local temperature may be overestimated by the MPS depending on the internal temperature of the circuit board containing the temperature sensors. The temperature of the circuit board increases with, e.g., the number of measurements configured and the duration of the measurements.

3 Strong peaks in the rpm data typically indicate a shaker imbalance. Check the weight distribution on your shaker, which should be balanced.

4 The rpm sensor provides reliable data above 50 rpm. At lower shaking speeds, rpm may be reported with reduced accuracy.

Available LED wavelengths and peak detector wavelengths

The LEDs located in the center of the central window are used for backscatter (biomass) measurements and excitation of fluorophores (see Figure 20 and Figure 21 for exact positions).

LED peak wavelength (typical)	Full width half maximum (typical)
385 nm	12 nm
415 nm	15 nm
465 nm	28 nm
496 nm	24 nm
530 nm	38 nm
560 nm	20 nm
574 nm	20 nm
590 nm	20 nm
622 nm	24 nm
645 nm	28 nm
850 nm	42 nm
940 nm	40 nm

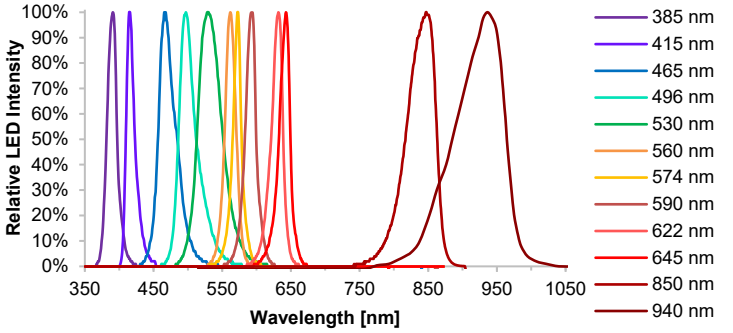


Figure 5: Spectra of LEDs used for backscatter and fluorescence monitoring. The intensity scale is relative to each peak maximum.

The detection spectra available on the MPS are listed below (see Figure 20 and Figure 21 for exact positions).

Peak detector wavelength (typical)	Full width half maximum (typical)
415 nm	19 nm
445 nm	21 nm
480 nm	25 nm
515 nm	28 nm
555 nm	27 nm
590 nm	25 nm
630 nm	32 nm
680 nm	36 nm
(Full backscatter) 930 nm	400 – 1100 nm detection range
(NIR backscatter) 880 nm	730 – 1100 nm detection range
(NIR spectrometer) 910 nm	850 – 1100 nm detection range

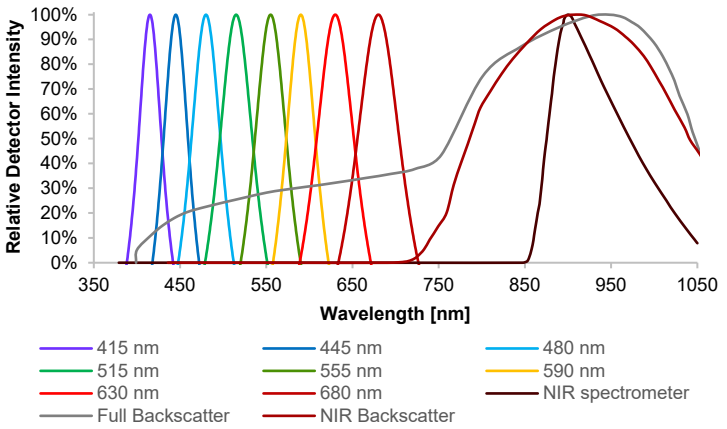


Figure 6: Detection spectra available on the MPS. The intensity scale is relative to each peak maximum.

DO Sensor Pills

Core material	PA-6
Form factor pill	
Volume	1.3 cm³
Density	1.25 g/cm³
Form factor marble	
Volume	1.8 cm³
Density	1.1 g/cm³
Sterilization	Beta irradiated at 25 kGy
Response time t_{90}[1-3, 6] (typical)	< 60 s
Shaking noise[4, 6] (typical)	
Peak-to-Peak	1 – 8 %
Standard Deviation	0.5 – 1.5 %
Accuracy (typical)	
5% air saturation[5]	4 %
95% air saturation[5]	2 %
Resolution[6] (typical)	
5% air saturation[5]	<0.2 mbar or 0.1 % air saturation
95% air saturation[5]	<0.2 mbar or 0.1 % air saturation
240% air saturation[5]	0.2 mbar
Measuring range[6] (typical)	0 – 470% air saturation[5]
Detection limit[6] (typical)	0 – 0.5 % air saturation[5, 6]
Drift (typical)	< 0.2 %/day[7]
Lifetime (typical)	>1 million data points
Shelf life (typical)	6 months in original packaging[8]

Storage conditions	Dry, dark, and at room temperature
Cross sensitivities	Organic solvents at high concentrations, bleach

Chemical compatibility[6,9] (typical)	**Compound**	**Maximum concentration[10]**
	Ethanol	10 %
	Methanol	5 %
	Ammonia	1 %
	Ammonium Chloride	1 %
	Acetic acid	5 %
	HCl	1 %
	NaOH	1 %
	Tris-HCl	50 mM

1 All measurement specifications represent typical values under commonly observed cultivation conditions. Each measurement specification may be influenced by cultivation, ambient and shaking conditions, compounds in the media, handling of Pills and devices, calibration codes provided to the DOTS Software as well as other factors or parameters unknown to the author.

2 Time to reach 90% of the equilibrium sensor signal during a step response from 100% DO in ambient air to 0% DO via sulfite addition. Recorded at 30°C in PBS, 250 ml shake flask with 10 % filling volume, at 300 rpm with 25 mm shaking diameter. The DO was changed instantly from air saturation to zero DO by catalyzed sulfite reaction (Cu^{2+} and sodium sulfite).

3 Response time may furthermore depend on agitation, temperature, pressure, medium polarity and other media and ambient conditions.

4 Shaking noise describes the maximum signal deviation from signal average during shaking.

5 Air saturation at 30°C, 1013 mbar air pressure, 0% humidity

6 Strongly depends on the shaking conditions as well as flask size, liquid levels, and dynamics

7 Drift given in percent deviation from initial DO Partial Pressure signal. Recorded under constant shaking, air saturation at 30°C, 100 % humidity, measured in PBS. Value refers to first week of continuous operation. The drift rate decreases after several days of operation.

8 A recalibration of the DO sensor pills may be required after prolonged storage (> 3 months).

9 The DO Sensor Pills are generally resistant to typical chemicals and typical concentrations found in microbial and cell cultures. The list only shows prominent examples. However, depending on the exact media composition, the chemical compatibility of the DO Pills may be different or cross-influenced by other compounds.

10 Compounds tested as additives in PBS. %-Concentrations are given in weight-percent.

Recommended operating conditions

MPS and Shake Flask Adapters (without Sensor Pills)

Temperature	4 - 45 °C (Ensure to let the MPS adjust to the operating temperature for 30 min.)
Humidity (relative)	≤ 85 % (non-condensing)
Shake flask filling volume (without Sensor Pill)	
optimal range	10 – 20%
good range[1]	5 – 25%
applicable range[2]	2 – 30%
extended range[3]	0 – 50%
Shaking speed (without Sensor Pill)	
optimal range[4]	50 – 300 rpm
shaking diameter ≤ 2.5 cm	0[4] – 350 rpm
shaking diameter ≤ 5.0 cm	0[4] – 300 rpm
Optimal performance[5] under ambient light[6]	

Biomass measurement	darkened shaker
Fluorescence measurement	darkened shaker
DO measurement	coverless

1 Measurement quality should be as good as for the optimal range, in few cases reduced precision or weak artifacts may be observed.

2 Measurement quality should be acceptable, in some cases reduced precision or artifacts may be observed.

3 Measurement quality can be acceptable, in many cases reduced precision or artifacts might be observed, filling volumes above 50% shouldn't be used to avoid spilling of the liquid during shaking.

4 Use shaking speeds within the optimal range for optimal measurement results. For other shaking speeds within the general specification range, in few cases reduced precision or weak artifacts may be observed. Measurements at speeds below 50 rpm require a firmware update, please contact our support team.

5 Optimal measurement performance regarding sensitivity at low cell densities, or fluorescence intensities, optimal signal-to-noise ratio and minimized number and size of measurement artifacts.

6 The MPS actively compensates ambient light. Depending on the application-specific ambient light and cultivation conditions, this compensation may be incomplete. Constant ambient light can be compensated efficiently by the MPS. Strong and fast changes of the ambient light intensity may be visible as step-like artifacts in the measurement data.

Sensor Pills – Dissolved Oxygen

Microbial Applications – Form factor: Pill

Temperature	4 – 45 °C
Shake flask type	Glass flask without baffles
Shake flask filling volume	
100 ml shake flask	10 – 20 %
250 ml shake flask	5 – 20 %
500 – 2000 ml shake flask	5 – 10 %
Shaking speed[1]	
Optimal range	200 – 300 rpm
shaking diameter ≤ 2.5 cm	200[2] – 350 rpm
shaking diameter ≤ 5.0 cm	180 – 300 rpm

1 For process conditions that include low rpm (<< 200 rpm), or other conditions that do not enable Pill movement with the liquid, Pills with a different form factor may enable DO measurements. Please contact our support team.

2 For 100 ml shake flasks, and 250 ml shake flask with less than 10 % filling volume, a minimum of 250 rpm applies.

Cell culture applications - Form factor: Marble

Temperature	4 – 45 °C
Shake flask type	Glass or plastic flask without baffles
Shake flask filling volume[1]	
100 ml shake flask	10 – 50 %
250 ml shake flask	5 – 40[2] %
500 ml shake flask	5 – 30[3] %
Shaking speed[4]	
Optimal range[5]	180[6] – 250 rpm
shaking diameter ≤ 2.5 cm	120[7] – 250 rpm
shaking diameter ≤ 5.0 cm	110 – 250 rpm

1 Applicable ranges of filling volumes may be extended for certain models of plastic shake flasks.

2 For 2.5 cm shaking diameter, a maximum of 32 % shake flask filling volume applies.

3 For 2.5 cm shaking diameter, a maximum of 20 % shake flask filling volume applies.

4 Shaking speed limits apply to the complete recommended range of filling volumes. Lower shaking speeds are possible with low filling volumes.

5 Optimal shaking speeds ensure a regular circulation of the marble in the shake flask liquid with optimal data quality. At lower shaking speeds, marbles may not follow the liquid in a regular pattern. While DO measurements are possible, the irregular marble movement in the shake flask potentially affects other measurements by the MPS.

6 For 100 ml flasks, a minimum of 200 rpm applies.

7 For 100 ml flasks, a minimum of 140 rpm applies.

Warnings

Do not use the MPS devices or any of its components in water bath! This may result in electric shocks, which could damage your health, the MPS device and any other electric device around.

Do not look into the light beam of the operating MPS. Their emitted light is of high intensity and might damage your eye or retina. Wear protective eye wear.

Do not touch, wet or electrically bridge the USB connectors! This might result in damage to your health and/or the MPS device.

Due to possible electromagnetic radiation, do not remain closer than 20 cm to the device for a longer period.

The device contains a lithium-ion battery. Take special care considering following aspects:

- **Do not dispose the battery / the device into normal waste.**
- **Do not heat or dispose the battery into fire, water or other liquids.**
- **Do not put the device into a microwave, washing machine or a drying machine.**
- **Never use a damaged battery.**
- **Never disassemble the device.**
- **Should a battery unintentionally be crushed, thus releasing its contents, rubber gloves must be used to handle all battery components. Avoid inhalation of any vapors that may be emitted.**

Take care when shipping the device. Inform yourself about local shipping regulations of lithium-ion battery containing devices.

Do not charge / discharge the battery outside the operating temperature range.

For longer storage of the device, the battery should be in a 50% charged state.

Do not spill liquids over the MPS devices. Liquids may enter the housing and destroy the device by corrosion.

Do not operate the system with flasks filled and/or operated in a way they can spill. Test each process condition before placing a flask on a sensor.

Do not use the system in a way that any of its components might get damaged. If you are using process conditions that are far away from the recommended settings in this user guide, test the conditions beforehand and/or consult our Application Scientists. Any damage caused from wrong usage, known or unknown, excludes the system from our warranty.

Do not reuse or sterilize Sensor Pills on your own. The measurement range, accuracy, and factory calibration are not longer applicable and pill functionality may be limited.

In general, do do not use inorganic or organic acids and bases, organic solvents or detergents to clean the MPS and Shake Flask Adapters or MPS Hubs. Some organic solvents or detergents might be allowed for cleaning, but you should only use those being mentioned in the user guide.

Any kind of opening, manipulating or copying MPS devices as well as decompiling, reverse-engineering, copying or distributing DOTS Software or firmware or its components is strictly prohibited in accordance with German and international law and may lead to compensation claims.

SBI sensors, devices and other equipment are not intended for medical or military purposes or any other safety-critical applications. It is strictly prohibited to use SBI sensors, devices and other equipment for applications in humans or for applications where sensors are brought in direct contact with foods, drinks, tissues or other goods that are transferred into humans.

Setup and Handling

Setup overview

A typical workflow to set up an experiment with the Multiparameter Sensor includes the following steps:

1. Setup the hardware components on the shaking tray.
2. Plan your Experiment in DOTS Software.
3. Prepare shake flasks (add DO Sensor Pill if required).
4. Install shake flasks inside the Shake Flask Adapters.
5. Start the Experiment via DOTS Software.

Detailed setup steps for the hardware are explained below. Information on all DOTS Software workflows can be found in the DOTS Software User Guide.

Shake Flask Adapters are directly attached to the shaker tray and used to position the shake flasks above the MPS. Adapters are available for glass shake flasks of nominal volumes 100, 250, 500, 1000, and 2000 ml, and for plastic shake flasks of 100, 250, and 500 ml. If special flasks are used, contact our support team for adequate Adapters. MPS Hubs are mounted on the shaker tray and connect up to 7 or up to 16 MPS per Hub to a computer outside of the shaker. For DO monitoring, single-use DO Sensor Pills are added in a separate step prior to each experiment.

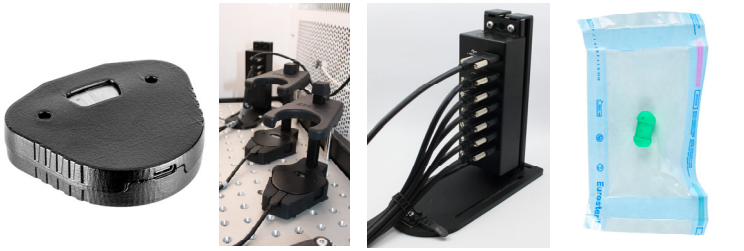


Figure 7: Components of the multiparameter sensing system: The MPS (Multiparameter Sensor), the Shake Flask Adapter holding the MPS with USB cable, the MPS Hub (7 MPS ports), and the single-use DO Sensor Pill.

Hardware setup on the shaker tray

Installing the Shake Flask Adapters

The Shake Flask Adapters exhibit elongated holes on their bottom, enabling installation on each kind of shaker tray using screws. When installing the Adapter, simply move it over the tray until screwing threads are visible through both elongated holes in the base center and through the silver plate on each side of the metal rod. Then, use appropriate screws that fit your tray's screw threads to fix the Adapter on the tray. For 100-500 ml Adapters, use 4 screws in total as shown in Figure 8. The plate in the back (indicated 1) uses one screw on each side of metal rod, the center of the base (indicated 2) requires two screws in total, one per elongated hole. For 1000 ml and 2000 ml Adapters, add a fifth screw on the additional elongated hole most distant from the metal rod (indicated 3).

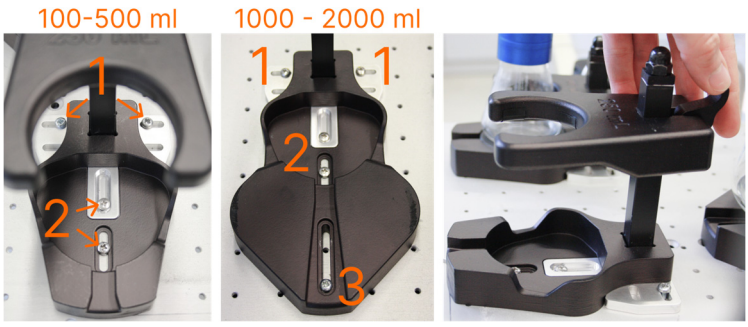


Figure 8: Shake Flask Adapter 250 ml (left) and 2000 ml (center) screwed into a shaking tray (1 –screws on the back plate, 2 – screws in base center, 3 – additional screw on large Adapters). The handle on the back of the Adapter ring (right) is lowered to slide the ring up or down.

Check that the screw heads fit well into the elongated holes on the Adapter bottom, otherwise they can damage the MPS that is installed on top of them.

Make sure to use the correct screws inside the Shake Flask Adapters, which fit well into the elongated Adapter bottom holes, to avoid damaging the MPS housing and USB cable.

An extra short screwdriver is supplied with Shake Flask Adapters, which facilitates the access of screws on the Adapter bottom. If you need more space to access the screw holes with the screwdriver, you can move the Adapter ring upwards. Move the handle on the back of the ring down, then move the ring to the desired position, and secure the ring by pushing the handle completely up.

Install as many Adapters as you need. Leave enough space between Adapters to be able to access all Adapter ring handles and flasks.

Installing the MPS Hub

The MPS Hub's bottom plate contains elongated holes, enabling its installation on each kind of shaker tray using screws. When installing the MPS Hub, simply move it over the tray until at least two screwing threads are visible through two elongated holes. Then use appropriate screws that fit your tray's screw threads to fix the MPS Hub on the tray. Ideally, and especially for high shaking speeds > 250 rpm, use one or more screws on each of the elongated holes.

Avoid the usage of countersunk head screws, as they might destroy the MPS Hub bottom plate when being screwed too tightly.

We recommend using a washer per screw for the MPS Hub installation. Doing this will reduce the mechanical load on the edges of the elongated holes and will thus increase the stability and lifetime of the MPS Hub bottom plate.

All USB-C cables on the MPS Hub must be fixed with the provided screws on the cable ends to avoid connection issues and damage to the equipment during shaking.

Each MPS Hub can connect multiple sensors and one computer (or another Hub). Always connect the MPS Hubs to a USB 3.0 (or higher) compatible port of your computer. The topmost port is used to connect the Hub to the PC outside of the shaker. Connect the USB-C to USB-A cable on this port and connect the USB-A elongation cable, which will reach outside the shaker. The other USB-C ports on the Hub are used to connect the MPS to the Hub. Install as many cables as required for all MPS. Secure all cables to the ports using the screws

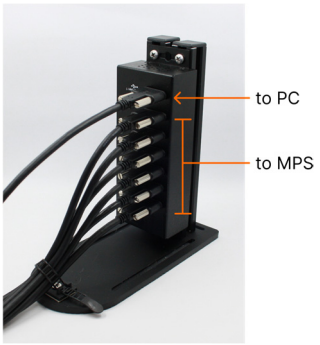
on the end of each cable. Then, secure all MPS cables using the preinstalled multi-cable management tool on the bottom plate of the MPS Hub as shown in Figure 9.

Figure 9: MPS Hub installed on the shaking tray. The topmost USB-C port is used to connect the Hub to the computer. The ports below connect the MPS devices to the Hub (in this case, 2 MPS).

Installing the MPS in the Shake Flask Adapter

The MPS fits tightly into the cavity on the base of the Shake Flask Adapter. Mount the MPS inside the Adapter cavity with the USB port facing away from the vertical rod of the Adapter, and make sure the sensor windows are facing upwards. Push the MPS down to the bottom in case it does not slide down on its own. However, avoid touching sensor windows.

Figure 10: Installing the MPS in the Shake Flask Adapter.



Connect a USB-cable from the USB-Hub to each MPS. Any port can be used for an MPS as long as the topmost port is correctly connected to the computer with DOTS Software.

Figure 11: Connecting the MPS to the USB cable from the MPS Hub.

Ensure that the MPS sensors are connected to the correct ports of the MPS Hub. The topmost port connects the MPS Hub to a USB 3.0 (or higher) compatible port on the computer.

All cables must be secured tightly onto the shaker tray. The following section explains in detail how to setup good cable management.

Cable routing in and outside of the shaker

Shaking movements create a constant mechanical load for the MPS USB and power supply cables, which must be minimized by suitable cable routing inside the shaker. All cables must be secured tightly onto the shaker tray to avoid movement during shaking. This applies especially to the connection ports, where any movement can cause connection issues or even damage the devices.

General cable management tools are provided with the MPS system. The MPS cables should be routed properly on the shaker tray to not hit the wall of the shaker while shaking and should be fixed on the tray using the screwable cable management tools (Figure 12).

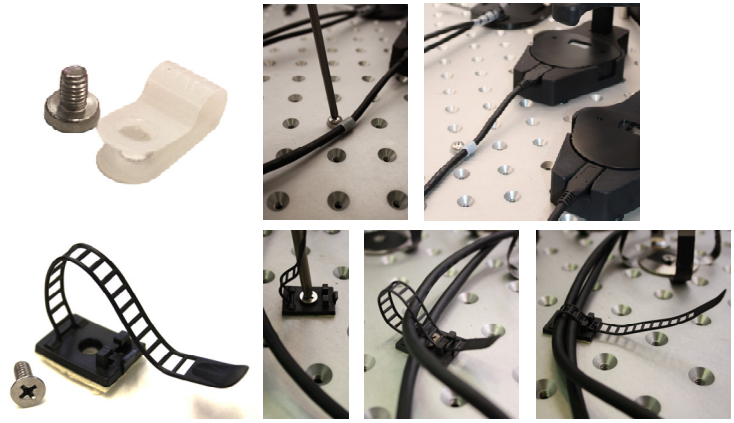


Figure 12: Single (top) and multi-cable (bottom) management tools for fixing cables onto the shaking tray.

The USB cable that is connected to the PC outside the shaker (attached to the topmost port of the USB-Hub) should be routed through the front door of your shaker or through side wall openings, as depicted in Figure 14. Use the cable clamp array as shown in Figure 13 and resize it according to your requirements. It can either be screwed or glued to position.

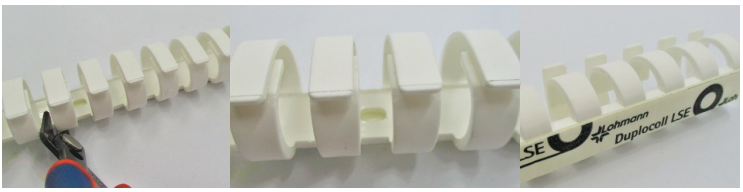


Figure 13: Cable clamp array customization (left), screw mounting hole (center) and sticky back (right).

Use the cable clamp array to guide the cable as depicted below. The cable must have enough slack between the point where it is held by the cable clamp array and the MPS Hub.

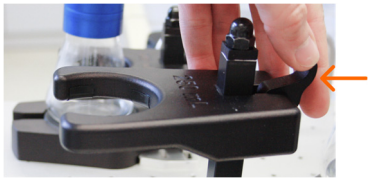
If no MPS Hub is installed and individual cables are used, the cables must be secured especially well near the USB port on the MPS. The end of the cable

that routes outside the shaker must have enough slack to not create tension during shaking.

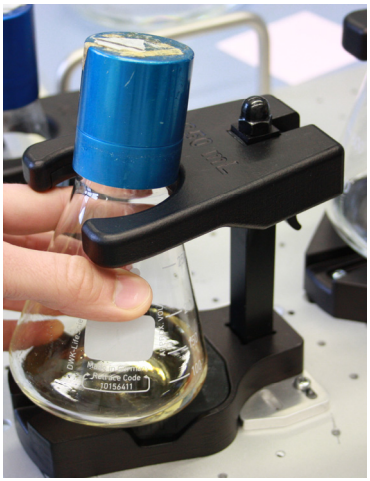
Figure 14: Cable management inside the shaker.

Installing shake flasks in Shake Flask Adapters

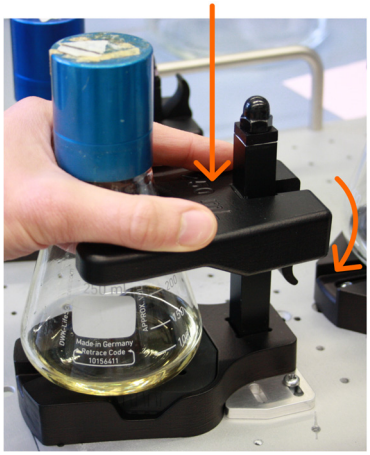
Prepare your flasks as usual. If you wish to monitor DO, include a DO Sensor Pill in each flask (p. 32).



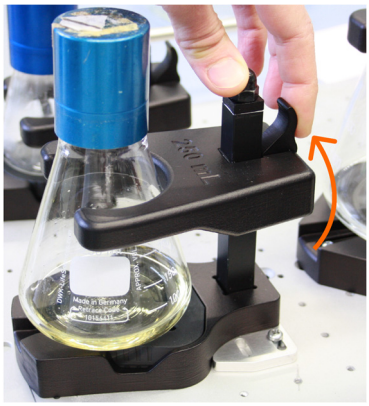
Lower the handle on the back of the Adapter ring to lift the ring upwards.



Place the shake flask onto the MPS, with the shake flask neck located inside the Adapter ring.



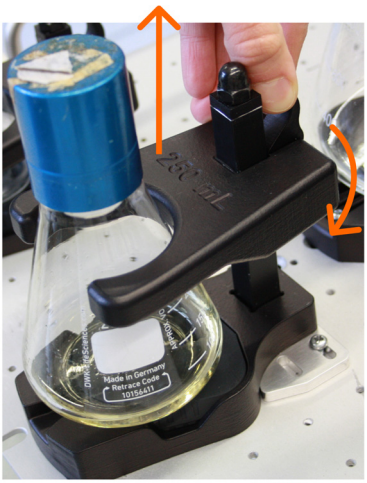
With the handle kept down, move the Adapter ring down until it embraces the shake flask.



Push the handle completely up to fix the ring in place.

Figure 15: Installing a shake flask in the Shake Flask Adapter.

Removing shake flasks from Shake Flask Adapters



Lower the handle on the back of the Adapter ring and lift the ring upwards until it is possible to remove the flask. In some cases, the flask neck might slightly stick to the Adapter ring, be careful and hold onto the flask while moving the ring upwards.

Secure the Adapter ring in place by lifting the handle completely up.

Figure 16: Removing a shake flask from the Shake Flask Adapter.

Connection to DOTS Software

The latest firmware should be installed on all MPS. Contact our support team for instructions on how to update device firmware.

After having successfully installed the MPS hardware on your shaker, you are now prepared to connect to the DOTS Software. For details on the DOTS Software, refer to our DOTS Software User Guide. Connect the cable from the topmost USB port of the MPS Hub to the PC that runs the DOTS Software. Upon first time connection, all MPS will shortly light up all LEDs (initialization). The MPS appear automatically on the DOTS Software Device list.

If you wish to rename individual MPS, click on them to open the Device Details. To identify individual MPS, disconnect them one by one on the respective MPS USB port and check which MPS shows as disconnected on the Device List.

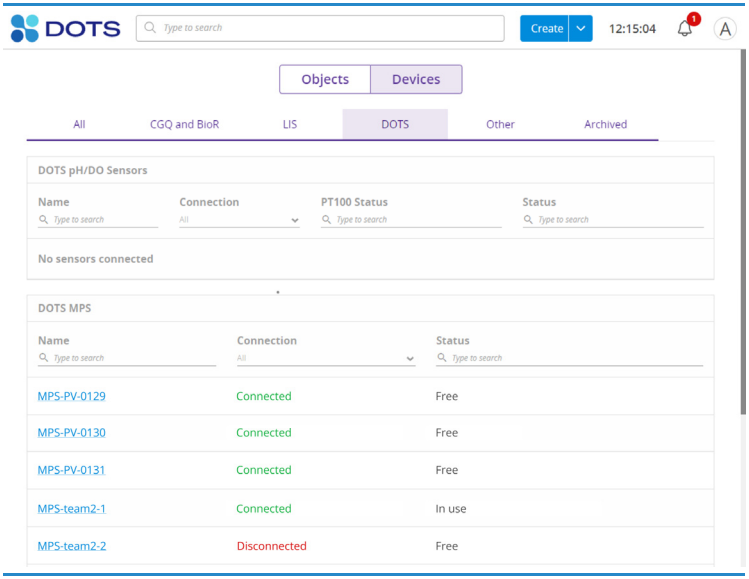


Figure 17: The DOTS Software device list.

Preparing flasks for DO monitoring

Different types of DO Sensor Pills are available for microbial applications (glass flasks) and cell culture applications (single use plastic flasks). All types of Pills are optimized for non-baffled flasks and typical process conditions of the respective application areas. Currently, baffled flask experiments are not fully supported due to mechanical wear at the Pill surface. While this usually does not compromise the DO measurement, you may observe the undesired detachment of paint-layers from the Pill when used in baffled shake flasks.

The pre-sterilized DO Sensor Pills must be handled in a sterile environment, such as a laminar flow hood, biosafety cabinet, or clean bench, to maintain sterility.

DO Sensor Pills are sterile products. Add them into your shake flask in a sterile environment to maintain their sterility.

Never re-use, and never sterilize the DO Sensor Pills by yourself. This will degrade the sensitive dyes, affect the calibration drastically and ultimately result in unreliable or even completely wrong DO data.

Microbial Applications: Use clean glass flask without scratches or dirt on their inside. These may provoke undesired detachment of paint-layers from the Pill caps.

Open the foil package containing a single DO Sensor Pill. Keep the package or save the sensor code shown on the package label (highlighted orange in Figure 18). This code contains a factory calibration that must be used when setting up a new experiment in the DOTS Software. Usually, no further user calibration is required. Then, simply drop the pill into your flask.

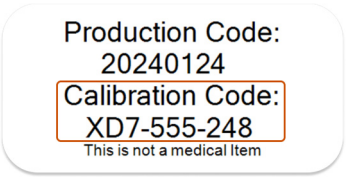


Figure 18: Sensor code located on the exterior of each DO Sensor Pill package.

To open the DO Sensor Pill package, tear the bag open across the labels with a quick move.

DO Sensor Pills must circulate with the shake flask liquid so that the MPS can read the pill's signal. Upon starting the shaker, verify that all pills in your experiment are circulating well in the shake flasks. This also applies after shaking has been interrupted and resumed during an experiment.

If you observe that DO Sensor Pills are not circulating at low shaking speeds, short-time increase the shaking speed to 250 rpm or higher. Once the Pills circulate, reduce the shaking speed again to your desired rpm.

A stable pill movement with the liquid is only possible if your liquid is moving in-phase with the shaking movement. Any out-of-phase condition will deteriorate the pill trajectory and prevent robust DO reading through the MPS. Below a certain critical rpm, the Pill circulation becomes irregular despite in-phase liquid movement. In such cases, the DO data quality will still be acceptable if the Pill passes by the shake flask wall and completes a full circle in less than 2 seconds. However, an irregular Pill and liquid movement can affect the reproducibility of experiments, as well as other optical measurements by the MPS. Recommended shaking conditions can be found on page 18.

DO Sensor Pills cannot be detected while shaking is paused, unless the pill is placed directly above the DO frontend. Pausing your measurement while shaking is interrupted removes artifacts from your DO signal. The MPS detects DO measurements under non-shaken conditions and label them as invalid.

Cleaning and Disinfection

Ensure that you have disconnected all MPS, MPS Hubs, and cables from any kind of power supply or PC, to prevent damages to the electronics, to connected devices, and to your health.

Ensure that all hardware and cables are completely dry after disinfection, before you reconnect them to each other and to the PC and MPS Hub.

The MPS housing must never be soaked in or sprayed with any cleaning or disinfectant liquids. No liquid must enter the housing to prevent permanent damage to the electronics. Do not apply pressure while wiping with a pre-wetted tissue.

Component	Cleaning	Disinfection
MPS Hub (except for bottom plate)	Gently wipe with (damp) lint-free wipes (e.g., Chemwipes)	Except for the bottom plate, wipe gently with 70 % ethanol wipes
MPS Hub bottom plate	Gently wipe with (damp) lint-free wipes (e.g., Chemwipes)	Special PMMA disinfectants, such as Bacillol® 30 Tissues
Shake Flask Adapters	Water and mild soap	Special PMMA disinfectants, such as Bacillol® 30 Tissues
MPS	The housing, excluding the sensor windows, can be gently wiped with a damp lint-free wipe	The housing, excluding the sensor windows, can be gently wiped with PMMA disinfectants suitable for sensitive surfaces, such as Bacillol® 30 Sensitive Tissues
MPS sensor windows	Wipe off dust from the sensor window with dry lint-free wipes (e.g., Chemwipes).	Use only pre-wetted tissues with PMMA disinfectants suitable for sensitive surfaces, such as Bacillol® 30 Sensitive Tissues
USB cables	Water and mild soap	Wipe gently with 70 % ethanol wipes

Basic measurement principles

Biomass monitoring

Optical cell density measurements

Noninvasive cell density monitoring in shake flasks is based on the principle of light scattering. Each measurement consists of a sequence of three major step: (1) Light is irradiated by a LED into the fermentation broth through the transparent vessel wall. While most of the photons go straight through the liquid, some interact with the cells and are scattered in different directions.

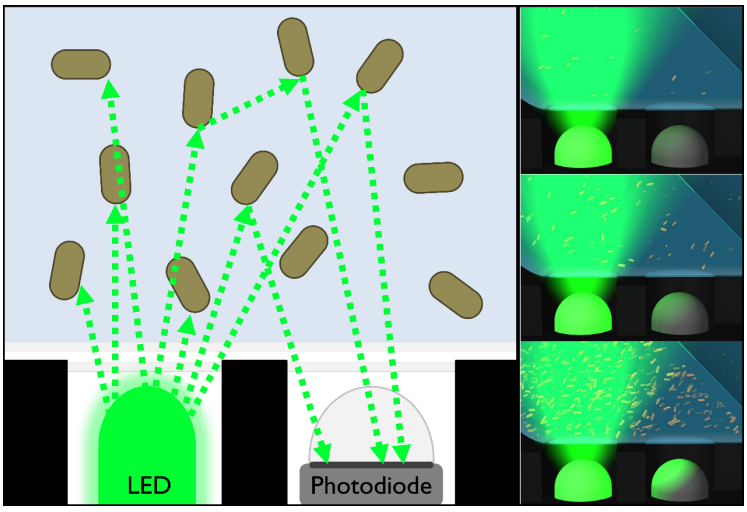


Figure 19: Optical cell density measurement using light scattering.

(2) The backscattered photons are detected by a photodiode, which converts the scattered light intensity into a weak electrical current. (3) This initial raw signal is subsequently amplified and subjected to various analytical algorithms on the MPS, yielding the current cell density. The higher the cell density in the fermentation broth, the higher the probability of an incident photon to interact

with a cell, so that with increasing cell density more light is scattered towards the photodiode.

Each MPS comprises highly sensitive sensor technology implemented around an array of LEDs and photodiodes. The MPS measurements of backscattered light allow for cell density measurements in the range of 0.1 OD_{600} and enable you to monitor fermentations that grow beyond 80 OD_{600}. In contrast to typical transmission-based spectrophotometers, the light scattering analysis implemented in the MPS guarantees cell density measurements over three orders of magnitude without any requirements for diluting or concentrating steps. The MPS system consequently provides you a simple, efficient, and accurate tool to monitor biomass concentrations of your shake flask and bioreactor fermentations noninvasively, and without the need for additional devices.

The MPS are equipped with technology and algorithms to distinguish between ambient light and the backscatter signal, thus allowing for ambient light compensation. This is essential for cell density monitoring in vessels, which cannot be covered and darkened as easily as shake flasks. The ambient light compensation is intended for operating conditions with constant or intermediately changing ambient light intensities. Strong intensity changes, especially with periods of absolute darkness, may cause step-like artifacts in the measurement curves. For optimal measurement performance, it may still be recommended to darken the shaker (i.e., cover the door window). Please refer to the recommended operating conditions (pp. 17),

The standard MPS peak wavelength used for biomass detection is 940 nm (infrared). The probability for infrared photons to be scattered by cells is lower than for photons at shorter wavelengths, which provides you with a unique opportunity to monitor the growth of higher cell density flask fermentations, e.g., of yeasts, with achievable cell densities of 30 OD_{600} (20 g/L) and beyond.

Furthermore, the excitation wavelength of each MPS is customizable via the DOTS Software Custom Templates. Depending on application-specific

requirements, you can choose your preferred wavelength together with our application specialists to avoid an interference of backscatter measurements with secreted or cellular chromophores or fluorophores, such as chlorophyll, heme proteins, GFP, etc. Extreme shaking conditions, such as < 5 % flask filling volume at 350 rpm, may also require adjustments of the LED choice.

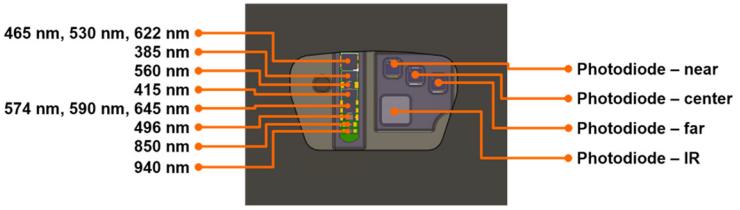


Figure 20: Positions of LEDs for illumination of the shake flask and photodiodes for backscatter measurement on the MPS. The top of the image is oriented towards the shake flask wall, the bottom towards the center of the flask.

Guidelines for high-quality biomass data

Default settings in the DOTS Software Biomass Application are functional for a wide range of typical shaking conditions, which can be found on p. 11. Conditions well outside these ranges, and culture media that contain strongly colored components or particles, may require adjustments of the detection settings.

LED position. The available LEDs on the MPS differ in position, as shown in Figure 20. The DOTS Software default settings use the 940 nm LED, which is located on the edge of the LED strip closest to the shake flask center. Conditions that move the shaken liquid closer to the shake flask wall may leave only a thin layer of liquid above the standard 940 nm LED (compare Figure 25). An example is the combination of low flask filling volume (< 5 %) with high shaking speed (300 - 350 rpm) at large throw (50 mm). Here, the use of an LED on the outer part of the LED strip, e.g., the 622 nm LED, will improve the backscatter measurements.

LED brightness. Our in-house LED calibration guarantees that equivalent LEDs emit the same level of brightness on each MPS. However, the level of brightness differs between the different LEDs on the LED strip (Figure 20). The 645 nm LED is the brightest. The 465 nm and 530 nm LEDs achieve the lowest brightness, which makes them less favorable for applications that require high sensitivity, such as low cell density cultivations or dark colored media.

LED wavelength. For most applications, the default 940 nm LED yields robust results. Its typical sensitivity lies in the range of 0.1-140 OD_{600} (0.06-80 g/L). The 940 nm LED is required for high cell density cultivations beyond 30 OD_{600} (20 g/L). For certain applications, the 622 nm LED may be more sensitive in the lower OD range (up to 1 OD_{600} or 0.6 g/L) than the 940 nm LED. Both can be tested in parallel with the standard Biomass detection settings in the DOTS Software. 622 nm or higher are required for biomass detection in presence of a DO Sensor Pill. Fluorescent or colored components inside the shake flask liquid may interfere with backscatter measurements when their absorption or emission spectra overlap with the spectra of the illuminating LEDs of the MPS. Detailed information on the LED spectra can be found on p. 13. For example, high concentrations of the red fluorescent protein mCherry (absorption maximum 587 nm) can enhance the backscatter signal performed with a 622 nm LED. In such cases, an LED wavelength outside of the mCherry spectra may reduce the influence of the protein presence on backscatter measurements (e.g. 940 nm LED).

Other internal settings of the MPS may be tuned to achieve high-quality biomass data for your application. Please contact our Application Scientists.

Correlation between OD_{600} and the MPS biomass signal

A typical measure for cell density is the optical density, often referred to as OD_{600} at a measurement wavelength of 600 nm and given by Lambert-Beer's law as

$$c = \frac{\log_{10}\left(\frac{I_0}{I_1}\right)}{\varepsilon_\lambda \cdot d} \quad \{1\}$$

with I_0 the incident light intensity, I_1 the transmitted light intensity, ε_λ the extinction coefficient at a given wavelength λ, c the concentration of the substance to be measured and d the transmitted path length. Widely known as Lambert-Beer's-law, this holds true only for homogenous dilute solutions, so that concentrations above 0.5 OD_{600} need to be diluted prior to transmission measurements and scaled accordingly after the measurement. The background of this range limitation is an increasing contribution of other optical effects, especially of multiple scattering, to the transmissivity, which is not described by Lambert-Beer's law.

As described above, the MPS measures backscattered light intensities. While scattering signals can be used to measure particle concentrations over several orders of magnitude, it must be noticed that there is no physical law to describe the correlation between cell density (OD_{600}) and scattering (MPS signals) as simply as is done for the transmission measurements with the Lambert-Beer law. Nevertheless, it is possible to describe this correlation using more elaborate empirical models to account for effects such as multiple scattering.

At higher concentrations (usually above an OD_{600} of 10) the MPS biomass signal exhibits a linear correlation with the actual cell density in the shake flask. Lower concentrations (usually below an OD_{600} of 5) give an MPS biomass signal that increases exponentially with increasing cell density. Between these two zones there is a transition zone, where the exponential correlation changes into a linear correlation. The DOTS Software implements offline sampling based estimation methods to correlate offline cell density data such as OD or Cell Dry Weight with the MPS's backscattered light signal. Additional information is provided in the DOTS Software User Guide.

Fluorescence monitoring

Fluorescence measurements

Noninvasive fluorescence monitoring in shake flasks is performed by an array of LEDs and a spectrometer sensor. Each measurement consists of a sequence of three major steps: (1) Light is irradiated by a LED into the fermentation broth through the transparent vessel wall. Fluorophores, such as fluorescent proteins, inside the shake flask absorb photons and re-emit light at a higher wavelength. (2) The emitted light from the fluorophore is captured by the spectrometer sensor, which converts the emitted light intensity into a weak electrical current. (3) This initial raw signal is subsequently amplified and subjected to various analytical algorithms on the MPS, yielding the current fluorescence intensity around a specific, user-selected wavelength.

The MPS are equipped with technology and algorithms to distinguish between ambient light and the fluorescence signal, thus allowing for ambient light compensation. The ambient light compensation is intended for operating conditions with constant or intermediately changing ambient light intensities. Strong intensity changes, especially with periods of absolute darkness, may cause step-like artifacts in the measurement curves. For optimal measurement performance, it is still recommended to darken the shaker (i.e., cover the door window).

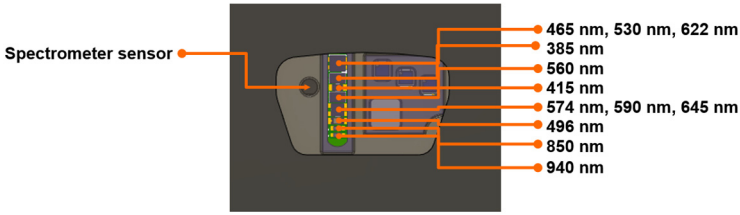


Figure 21: Positions of excitation LEDs for illumination of the shake flask and the spectrometer sensor for fluorescence measurement on the MPS. The top of the image is oriented towards the shake flask wall, the bottom towards the center of the flask.

Various excitation wavelengths are available on each MPS (Figure 21). These are the same LEDs that are used for backscatter measurements (p. 35). The spectrometer sensor located near the LEDs detects fluorescent light at various wavelengths. Details on the available excitation and emission wavelengths are described on p. 13.

Guidelines for successful fluorophore detection

Default settings in the DOTS Software Fluorescence Application are optimized for the detection of eGFP. For other fluorophores, and especially such with unknown fluorescent properties, some preliminary experiments are required to achieve optimal fluorescence detection. This section provides guidance on the tuning of MPS settings as well as important parameters that may influence your measurements. Contact our Application Scientists for support in finding good settings for your fluorescence application.

Selection of suitable excitation and detection wavelengths

Fluorophores absorb and emit light best at distinct wavelength peaks. In the DOTS Software, you should look for excitation and emission wavelengths close to the expected excitation and emission peaks of your fluorophore. However, choosing the closest fit might not always be the best choice.

Take into consideration that both excitation and emission spectra of a fluorophore are just that: spectra – with typical peak widths and intensity distributions. Furthermore, excitation and emission peaks usually overlap. The same applies to the LED and detector peaks of the MPS. Hence, suboptimal choice of MPS settings can lead to crosstalk between excitation, emission peaks, and even backscatter from the cells in the fermentation broth.

Figure 22 shows the detection settings for the popular fluorescent protein eGFP, as an example. The peak of the 465 nm LED covers a good portion of the eGFP excitation spectrum. Using the 555 nm data series for detection results in almost no crosstalk between the excitation LED and the detection peak. Choosing the 515 nm detection wavelength in this case – although the detector peak is closer

to the eGFP excitation peak - would result in too much crosstalk, and probably a visible influence of cell growth on the fluorescence signal.

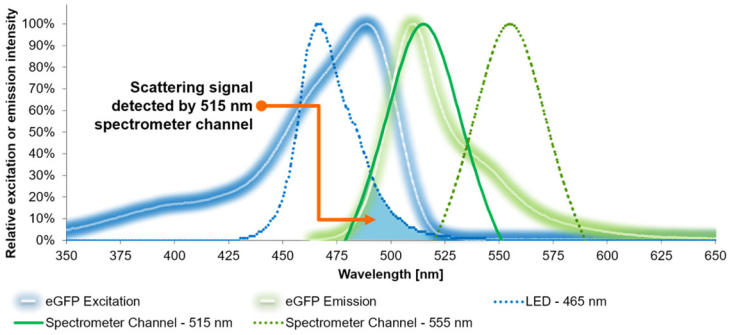


Figure 22: Exemplary guideline to select excitation and emission configurations of the MPS for eGFP[1]. A combination of the 465 nm LED with the 555 nm spectrometer channel provides sufficient eGFP excitation and emission spectra coverage while preventing scattering interference due to overlapping LED and spectrometer channel spectra.

Of course, the picture is not always as clear as in this example. If you are not sure which LED wavelength to select, or if you have a novel, uncharacterized fluorophore, you can screen for optimal settings using the DOTS Software. All available LED wavelengths can be recorded in one experiment. The measurements do not influence each other since they are performed quickly, one after another.

In the DOTS Software, you can screen the full excitation/emission spectrum. All available detection (emission) wavelengths can be combined with each configured LED (excitation) wavelength.

[1] Spectra data exported from fluorescent protein database: Lambert (2019) FPbase: a community-editable fluorescent protein database. Nature Methods 16, 277–278. doi: 10.1038/s41592-019-0352-8. Primary eGFP spectra source: Cormack at al. (1996) FACS-optimized mutants of the green fluorescent protein (GFP), Gene 173(1), 33-38, doi: 10.1016/0378-1119(95)00685-0.

High sensitivity fluorescence detection mode

DOTS versions 2.0.5 and newer offer a mode for high sensitivity fluorescence detection. This mode activates two LEDs for increased illumination: the user selected LED wavelength, and in addition, the LED of the next lower wavelength. For example, selecting the 560 nm LED as excitation light source will simultaneously activate the 560 nm and 530 nm LEDs. Furthermore, the high sensitivity detection mode uses a faster measurement procedure. This allows the MPS to capture more fluorescence light compared to the standard detection mode (within equivalent measurement duration).

Optimizing MPS fluorescence detection settings

A general challenge is the typically low intensity of fluorescent light compared to, e.g., ambient light in a shaker. Furthermore, higher wavelengths have a generally reduced light intensity despite their visibility by the human eye. As an example, red or far-red fluorescent proteins are far more difficult to detect than a green fluorescent protein, although they may appear just as bright by eye. However, carefully chosen settings may enable the MPS to detect even tricky fluorophores.

A fluorescence measurement on the MPS can be tuned by the two main parameters "**Spectrometer acquisition time**" and "**Spectrometer integration time**". The spectrometer acquisition time defines the overall duration of measurement. The exciting LED will appear switched on during the whole acquisition time. The longer the acquisition time, the more light is captured, and the higher the overall fluorescence signal will turn out. Within one acquisition time, the sensor flashes multiple times, each time as long as one integration time. This is done to identify the optimal measuring position, i.e., the timepoints at which the bulk of the shaken liquid is located on top of the sensor, and to reject ambient light. A short integration time can improve the signal-to-noise ratio, especially under stronger ambient light situations; however, the overall signal intensity is also reduced. As a rule of thumb, low integration times should be balanced with prolonged acquisition times. However, the overall suitable ranges of integration and acquisition times strongly depend on the properties of

the target fluorophore, the cultivation media, shaking conditions and ambient light levels. Not all fluorophores are equally straightforward to detect. Different excitation and emission peak widths, quantum yields, but also shaking conditions, are only some of the factors influencing fluorescence detection. Usually, the higher the excitation and emission wavelengths of a fluorophore, the harder it gets to capture enough emission light, i.e., higher acquisition time should be selected. The table below gives some examples of suitable settings that have been shown to work for the given conditions (all measured in 250 ml glass flasks).

Fluorophore	Conditions	MPS settings
eGFP (expressed by E. coli)	10-20 % filling volume, 250 rpm @ 25 mm throw	Ex/Em: 465nm/555nm Integration time: 100 msec Acquisition time: 6 sec
Dsred E5 photo-switch, green fluorescent state (expressed by E. coli)	10-20 % filling volume, 200 rpm @ 25 mm throw	Ex/Em: 465nm/515nm Integration time: 50 msec Acquisition time: 10 sec
Dsred E5 photo-switch, red fluorescent state, fully maturated (expressed by E. coli)	10-20 % filling volume, 200 rpm @ 25 mm throw	Ex/Em: 530nm/590nm Integration time: 100 msec Acquisition time: 30 sec
mCherry[1] (expressed by E. coli)	10-20 % filling volume, 200 rpm @ 50 mm throw	Ex/Em: 530nm/630nm *High sensitivity fluorescence mode* Integration time: 100 msec Acquisition time: 30 sec

Fluorescein < 0.2 µM – 40 µM (10 % Ethanol, 2 mM Tris-HCl pH 8)	10 % filling volume, 200 rpm @ 50 mm throw	Ex/Em: 465nm/515nm Integration time: 50 msec Acquisition time: 20 sec
Rhodamine B < 0.4 µM – 40 µM (10 % Ethanol, 2 mM Tris-HCl pH 8)	10 % filling volume, 200 rpm @ 50 mm throw	Ex/Em: 530nm/590nm Integration time: 50 msec Acquisition time: 30 sec

All measurements were performed in 250 ml glass flasks.

1 Louisa Kauth (2022): Development and establishment of photoswitchable systems on the surface of plant virus particles. PhD Thesis, RWTH Aachen University, Germany, https://doi.org/10.18154/RWTH-2022-09870. Construct pETDuet-Zdk1-G4S-mCherry-his6 was kindly provided by the Institute for Molecular Biotechnology, RWTH Aachen University, Germany

Other internal settings of the MPS may be tuned to achieve detection of low light emitting fluorophores or low concentrations of fluorophores. Please contact our Application Scientists.

Factors influencing fluorescence measurements

Biomass. An important aspect of fluorescence measurements in bacterial or cell cultures is the influence of biomass, or, more exactly, light scattering particles. Scattering particles reflect light and may generally enhance the fluorescence signal captured by the MPS. In addition, biomass present in the shake flask will scatter some of the (excitation) light from the LED back to the spectrometer sensor. This backscattered light may be bright enough to be captured by the sensor and add up to the fluorescence signal, especially when excitation LED and emission wavelength are close to each other, and the LED/detection spectra overlap a lot (as indicated in Figure 22). A measure to reduce the influence of biomass on the fluorescence signal is therefore to select excitation and detection wavelengths with small to no overlaps.

DO Sensor Pill. A DO Sensor Pill circulating within the bulk liquid may reduce the overall Fluorescence signal, since the light path available for fluorescence measurement in the liquid is partially occupied by the Pill. The reduced signal

will mainly occur for fluorophores of low fluorescence intensity, such as red fluorophores. To reduce this effect, integration times can be shortened while strongly prolonging acquisition times.

Shaking conditions. Shaking conditions can vary greatly between different bioprocesses. Any measure that enhances the amount of liquid on top of the MSP spectrometer can possibly improve fluorescence detection. Examples include increasing filling volume and flask size or decreasing shaking speed and shaking throw.

Other fluorophores or light absorbing compounds. Excitation/absorption and emission peaks of other fluorophores or light absorbing compounds may overlap with chosen excitation LEDs and/or detection channels. This can be reflected in either a reduction or enhancement of the measured fluorescence signal of the target fluorophore. In some cases, modifying the excitation and emission wavelengths to slightly suboptimal wavelengths for one fluorophore might avoid the influence of another fluorophore.

Ambient light. MPS fluorescence measurements are equipped with an ambient light compensation. However, sharp changes of ambient light intensity, and high ambient light intensities in general, such as in-built shaker lights, may still influence sensitive measurements. We recommend darkening the shaker by covering the glass window or even cover individual flasks. Contact our Application Scientists for more information on possible solutions against strong ambient light (changes).

Dissolved oxygen monitoring

The DOTS Platform offers dissolved oxygen (DO) monitoring in shake flasks using a patented technology based on Sensor Pills that circulate in the cultivation medium. Such DO Sensor Pills are made of a plastic core that is coated with an oxygen-sensitive luminescent dye. Depending on the surrounding oxygen concentration in the bulk liquid, the dye's luminescence is quenched, resulting in oxygen-dependent luminescence intensities and lifetimes, which are measured by the MPS as depicted in Figure 23. Red light is irradiated by the MPS into the shake flask in short flashes. Whenever the DO Sensor Pill enters the MPS-DO-frontend's field-of-view through the shaken liquid movement, the oxygen-sensitive dye is excited and it's oxygen-influenced near-infrared luminescence is read out by the MPS. With increasing oxygen concentration, the sensing dye's luminescence is increasingly quenched, resulting in lower luminescence intensities and lifetimes, which are then correlated with the dissolved oxygen concentration. Measuring from below the flask ensures that the DO Sensor Pill is always in contact with the bulk liquid to prevent measurement errors from air contacts of the sensing layer as usually observed with DO sensing patches that are glued onto the shake flask bottom.

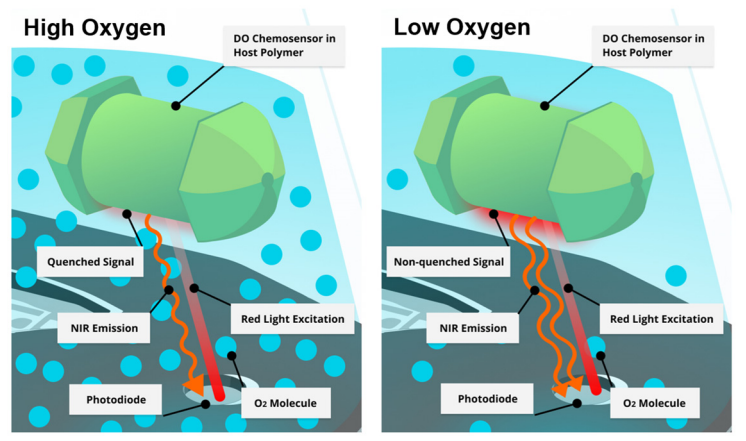


Figure 23: Working principle of the DO Sensor Pills.

Being sterilized by beta-irradiation, the DO Sensor Pills are single-use products and pre-calibrated for direct use. The calibration code is printed onto the Sensor Pill packaging and needs to be entered into the DOTS Software by the user. While the pre-calibration data are collected at 30°C, the dissolved oxygen readings are temperature-compensated by the MPS so that a recalibration is usually not required for common cultivation conditions. However, depending on the individual bioprocess conditions, the use of different calibration codes may be required to account for special media components, non-standard operating temperatures or specific lighting environments. Adjustments of the individual parts of the Sensor Code (Figure 24) may improve DO measurements in such conditions. For instance, using a high LED intensity (second digit = H) is advised for cultivations with expected high cell densities, cell aggregate formation, and dark media. Please contact our support team for further information.

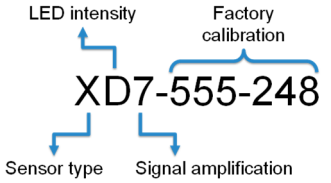


Figure 24: Types of information in the DO Sensor Code. The first three digits specify the detection settings, which are: Sensor Type: X = DO Sensor Pill; LED intensity: A-H (A = lowest, H = highest); Signal amplification: 1-7 (1 = lowest, 7 = highest). The center and last three digits contain the factory calibration that converts the raw signal to dissolved oxygen data (DO partial pressure; DO Percent (air saturation)).

DOTS Sensor Pills are the prerequisite for multiple optical measurements in a miniaturized form factor, so that monitoring of biomass, fluorescence, DO and other future parameters is possible even in a small 100 ml shake flask. The only requirement for getting robust sensor data in such a multiparameter setup is a stable in-phase movement of the liquid inside the shake flask under shaking conditions, allowing the MPS to detect and distinguish liquid, pills, gas and other phases through fast, angular correlated measurements. While the Sensor Pills are designed to maintain stable Pill trajectories under a broad range of shaking conditions, it is critical to operate the shake flasks under in-phase conditions.

The specified range of filling volumes and shaking speeds provides a basis to estimate these conditions (see page 18), however, depending on the individual shake flask process conditions, different optimal parameters may apply, especially regarding the combination of shaking speed, shaking throw, flask size, filling level, temperature, liquid viscosity and evaporation. The commonly known phase number can be used to predict whether a shaken system will be in-phase under certain bioprocess conditions[2].

Notes on DO Sensor Pills for cell culture applications

DO Sensor Pills with a marble form factor are designed for exclusive use in cell culture applications. They are currently optimized for non-baffled plastic flasks (e.g., Nalgene®, Corning®, Thomson Optimum Growth®). A few notes must be considered when using them in cell culture experiments that typically run for prolonged periods of time, i.e., several days to weeks. Please read the following paragraphs carefully prior to analyzing your online cell culture data and related offline measurements.

Shear stress. DO Pills can induce shear stress on your cell culture. This does not necessarily influence the culture's performance. However, if the shear stress from DO Pills is combined with other stress factors, a (usually reproducible) reduction in total cell counts, viable cell counts, and/or expression levels may be observed. Such additional stress factors include high shaking speed, uneven shaking (shaker imbalance), out-of-phase shaking, expression of a product, a culture recovering from cryopreservation, sampling and non-shaken transportation and storage, and senescent cells, among others.

[2]Amizon Azizan, Michaela Sieben, Georg Wandrey, and Jochen Büchs. „Reassessing the Out-of-phase Phenomenon in Shake Flasks by Evaluating the Angle-dependent Liquid Distribution Relative to the Direction of the Centrifugal Acceleration". *Biotechnology and Bioengineering* 116, Nr. 11 (November 2019): 2983–95. https://doi.org/10.1002/bit.27132.

Offline cell counts. Small pieces of the DO-sensitive paint may detach from the Pill surface over time due to friction between the Pill and the shake flask wall. Increased wear can be observed under certain conditions, such as prolonged use (> 1 week), high shaking speeds, and plastic flasks with particularly rough inner surface. While this wear will usually not compromise the DO measurements, microscopic paint flakes in the medium may be in the range of the cells' dimensions. Furthermore, the paint flakes are stained by live/dead assays such as trypan blue staining. Hence, the presence of paint flakes may affect the results of automatic cell counters using optical technologies (overestimating total cell count or underestimating viability).

Optical shake flask monitoring

Challenges in shaken vessels

Dynamic and heterogeneous liquid distributions are a key characteristic of shake flask cultivations. Performing highly accurate optical measurements under conditions of continuous shaking is therefore not trivial. Especially the huge variance of observable liquid distributions, which depend on a variety of different factors (e.g. flask size, flask shape, liquid volume, shaking speed, shaking diameter, liquid viscosity, temperature, etc.), requires adaptive sensor systems and measuring methods. The MPS combines these two aspects with extensive data analysis to allow robust and highly sensitive optical measurements under a broad variety of shaking conditions.

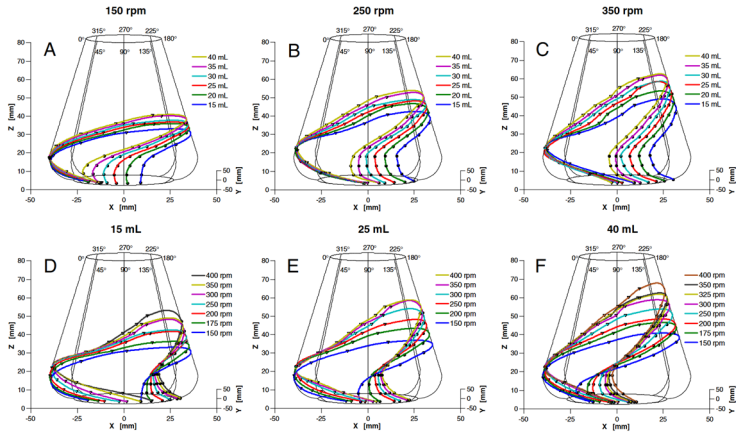


Figure 25: Illustration of the 3D-distribution of the bulk liquid under different shaking and filling conditions in a 250 ml non-baffled flask.[3]

[3] Figure reprinted under CC-BY 4.0 license (https://creativecommons.org/licenses/by/4.0/) from Amizon Azizan and Jochen Büchs. „Three-Dimensional (3D) Evaluation of Liquid Distribution in Shake Flask Using an Optical Fluorescence Technique". Journal of Biological Engineering 11, Nr. 1 (December 2017): 28. https://doi.org/10.1186/s13036-017-0070-7, Fig. 5

Using patented techniques for liquid tracking with time- and angle-resolved data acquisition in combination with advanced data processing algorithms, the MPS does not treat moving-liquid-induced signal fluctuations as noise, but as a source of valuable information. For determining one optical value, the MPS collects and fuses information from multiple different data types, including high-resolution time, acceleration, scattering as well as other optical and non-optical data. While such data fusion and processing enables monitoring of multiple important bioprocess parameters under continuous shaking conditions, optical MPS measurements, such as biomass (via backscattering), fluorescence or dissolved oxygen (via fluorescence) may be influenced by different ambient or otherwise bioprocess-related parameters, as described in the following.

Parameters influencing optical measurements in shake flasks

Each MPS is an optical measuring platform designed to provide the highest sensitivity and precision in the detection of scattered light, even at low particle concentrations.

In most applications, scattered light is used to measure biomass concentration. Depending on the chosen LED wavelength, the MPS backscatter data can contain additional information. The shorter the LED wavelength, the higher the probability that small structures and morphological changes are recognized - in addition to the whole cell scattering effect[4]. Small structures could be organelles, such as mitochondria or vacuoles in yeast cells, or bacterial endospores. Furthermore, inhomogeneities in the culture, such as different shapes and sizes of subpopulations, can influence the scattered light properties. Thus, it is important to acknowledge that the choice of LED wavelength can influence the backscatter measurement - especially in the

[4] Paul Latimer. “Light Scattering and Absorption as Methods of Studying Cell Population Parameters”. Annual Review of Biophysics 11:129-150 (1982). https://doi.org/10.1146/annurev.bb.11.060182.001021.

shorter wavelength ranges - when small cell sizes or cell substructures are prominent in the observed culture.

For comparable biomass measurements, keep the LED wavelength constant. Backscatter data recorded at shorter LED wavelengths (< 600 nm) have a higher probability to contain additional information / artifacts caused by scattering of small cell substructures or morphological changes and inhomogeneities in a culture.

The high sensitivity of the MPS backscatter measurements mean that changes in the optical environment will be detected by the MPS. With the ambient light compensation on the MPS, it is possible to monitor growth without the use of covers. To provide a reproducible optical environment for optimal measurements in shake flask applications, it is sometimes recommended to darken the shaker, as this creates a constant ambient light zone above the MPS, comparable to the dark measurement chamber of a spectrophotometer.

When comparing the real operating conditions of the MPS system with those of a typical spectrophotometer, some important differences should be noted.

Shake flasks are <u>not</u> a <u>cuvette</u>!

While cuvettes typically exhibit polished and perfect surfaces, a shake flask bottom or bioreactor side wall usually contains refraction anomalies from the production process and might be scratched to different extents. Such anomalies or scratches in the transparent vessel wall do not generally disrupt the MPS measurements. However, they might be an additional source of scattered light, influencing the comparability within a single and between several cultivations.

1. Within a single cultivation there is generally no influence on the data quality as long as the sensor-to-vessel position or orientation is not changed. If the flask or sensor is removed and remounted in a different position/orientation, there might be a different set of anomalies or scratches in front of the sensors, resulting in jumps in the cell density curve.

Try to avoid removing and remounting of the shake flask or MPS during cultivation. If this cannot be avoided, try to remount the system always in the same position/orientation as before, to avoid jumps in the cell density curve.

2. Between several cultivations, it is typically evident that different flasks are used. As each flask is individual regarding the number, size, and distribution of anomalies or scratches, the scattering intensities at equal cell densities differ between each flask. As long as the flasks' positions or orientations are not changed during the cultivation, these individual flask differences will (in most cases) only cause offsets in the MPS signal, which can be removed using the auto offset feature in the DOTS Software (Graph configuration, refer to the DOTS Software User Guide). Flask position/orientation changes during cultivation have been described above and should be avoided.

If a high comparability between several shake flasks is required, try to use flasks with similar degrees and distributions of scratching. In some cases, it may be favorable to preselect a set of highly similar flasks, based on measuring their scattering signal on the same MPS, at the same cell density, and at equal shaking conditions.

Shake flasks are <u>not</u> a <u>blank</u> cuvette!

While cuvettes typically exhibit blank surfaces, shake flasks and bioreactors are usually covered by volume markings, engravings, and labeling areas. Even if inscriptions are not located directly in front of the sensor window, they can act as an additional source of scattering if located in the sensor array's field of view. This scattering might interfere with the desired scattering of cells in the fermentation broth and may cause artifacts in the cell density curves.

Always ensure that the MPS is positioned and oriented in a way that no markings, labeling areas, or engravings are located in the sensor array's field of view.

Shake flasks are <u>not</u> an <u>even</u> blank cuvette!

While cuvettes typically exhibit even surfaces, shake flasks and bioreactors can be equipped with baffles, emanating either from the side walls or from the bottom of the vessel. Baffles have a curvy geometry, which might act as an

additional source of scattering, or as a lens, if located in front of the sensor array. This scattering might interfere with the desired scattering of cells in the fermentation broth, thus causing artifacts in the cell density curves. Furthermore, it should be noted that remounting the flask during a running cultivation might result in jumps in the cell density curve, similarly as described above for the scratches. Calibration data, if required, should always be collected per flask type.

Always ensure the MPS is positioned and oriented in a way that no baffles are located in front of the sensor array. If the vessel and baffle geometry permits such an orientation, then try to find a position/orientation so that the outer parts of the sensor array are not covered by the baffles.

Shake flasks are not an even blank quartz cuvette!

While precision cuvettes are typically made of quartz or high-quality glass, a shake flask can be made of glass or plastics, being clear, turbid, or colored; single- or double-walled. Generally, this is not problematic for the optical measurements, however, different materials might have different effects on the MPS signal. As long as the material is at least partially transparent for visible light, the MPS measurement principle will work, but care must be taken about some limitations.

1. Turbid materials reduce the amount of light going into the flask from the LEDs as well as the light leaving the flask towards the photodiodes, which results in reduced sensitivity. Turbid materials furthermore act as a strong additional scattering source, thus causing a much stronger background signal.
2. Colored materials may reduce the transmission of light into and out of the shake flask or vessel, similarly to turbid materials. However, the transmissivity of transparent colored materials is usually much higher than that of turbid materials, so the sensitivity reduction is not as strong as for turbid materials. Nevertheless, applying colored materials or cultivation media with strong absorption in the range of 500 – 550 nm might be difficult, and the use of different LED

wavelengths than in the standard MPS configuration should be considered (available LED wavelengths see p. 13).

3. Some typical plastics being used in single-use flasks exhibit anisotropic structures on a molecular level, which might act as an additional scattering source, resulting in increased background signal and cell density curve artifacts.

Generally, it is recommended to be careful when interpreting and comparing results from shake flasks made of different materials, as they might exhibit different background signals, sensitivities, or artifacts. Background correction can be done in the DOTS Software (Graph configuration, refer to the DOTS Software User Guide).

Always be careful when interpreting and comparing non-calibrated results from shake flasks made of different materials, as they might exhibit different background signals, sensitivities, or artifacts.

Shake flasks are <u>not</u> an <u>unagitated</u> even blank quartz cuvette!

While cuvettes are typically mounted in an unagitated environment, a shake flask is continuously shaken during the measurement, resulting in a continuously moving fluid. All fluidic parameters of these agitated systems, such as filling level, flask or vessel size and shape, shaking or stirring speed, aeration rate, flask cap, inline probes or sampling ports, fermentation broth viscosity, etc. might therefore influence the dynamically measured MPS signal (refer to pp. 51). The MPS data analysis algorithms are made and continuously improved (via firmware updates) to eliminate as many of these signal-influencing fluidic parameters as possible. However, you should not assume that the data from a 2000 ml flask, filled with 400 ml of broth and shaken at 150 rpm can be compared directly to a 250 ml flask, filled with 12.5 ml of broth, and shaken at 350 rpm. If you want to do direct and especially quantitative comparisons, calibrations are required as described in the DOTS Software User Guide.

Always be careful when interpreting and comparing non-calibrated results from shake flasks with different fluidic parameters (e.g. filling level, size, shape, agitation speed, cap, fermentation broth viscosity, etc.), as they might exhibit different background signals, sensitivities, or artifacts.

Always be careful when interpreting and comparing results from shake flasks, which have been subjected to filling volume changes (e.g. by sampling, feeding or induction). As long as the volume changes are smaller than 20% of the initial filling volume, the effects on cell density curves should be negligible, but larger volumetric changes might result in different background signals, sensitivities or artifacts.

Shake flasks are <u>not</u> an unagitated even blank quartz cuvette representing a <u>steady state system</u>!

While cuvettes are typically filled with a steady state solution as a kind of "current culture snapshot", a shake flask under cultivation is a highly dynamic and complex biological system, which changes continuously over time. These changes can include aspects influencing the fermentation broth's scattering behavior, e.g. filamentous growing organisms can change the viscosity, lipid secreting cells can create biphasic systems, strong morphologic cell modifications like sporulation can change the scattering diameter and secretion of colored substances, secretion or generation of aggregates, cell clumping, biofilm formation, etc. can furthermore contribute to the measured scattering signal.

Always be careful when interpreting and comparing results from cultivations, which are subjected to growth associated changes of parameters that influence the fermentation broth's scattering behavior. Those cultures may exhibit different background signals, sensitivities, or artifacts, depending on their respective growth behavior.

Troubleshooting

Common problems & solutions

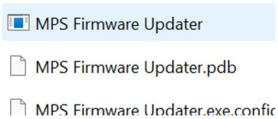
Problem	Solution
The MPS do not show up in the DOTS Software or do not connect at all	• Check if all Ports on the USB-Hub are connected correctly. The cable that connects the Hub with the computer must be connected to the Hub port labeled “power”. • Check if the LED “power” on the Hub is lit (green). If not, connect the USB cable without extension cable directly to the computer and see if the problem is solved (extension cable broken). • Log in with a DOTS admin account and verify that the device has been made available to your DOTS Team.
No data are coming in although measurement is running	Depending on the Sensor Firmware, shaking movement at a certain minimum rpm (typically 120 rpm) may be required to activate the measurement. Start the shaker and see if data are updated during shaking.
DO data are always zero or close to zero	• Check if the DO Sensor Pill is circulating with the shaken liquid inside the flask, close to the vessel wall. If not, increase the shaking speed to 250 rpm or higher until you see that the Pill is circulating. Then lower the shaking again to your desired rpm. • Check if your shaking conditions meet the requirement (p 18). You might

	need to adjust the rpm (increase) and/or filling volume. • Check if the DO Sensor windows and the flask on top are clean
The DO Sensor Pill is not circulating with the liquid inside the shake flask	• Increase the shaking speed to 250 rpm or higher until you see that the Pill is circulating. Then lower the shaking again to your desired rpm. • Check if your shaking conditions meet the requirement (p 18). You might need to adjust the rpm (increase) and/or filling volume.
The MPS keep blinking when disconnected and no experiment is running	• The MPS runs on battery when disconnected from the PC. The blinking patterns inform about idle and/or charging states (p. 62).

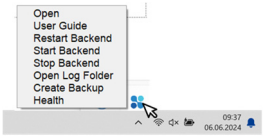
Updating the MPS firmware

Contact our support team to get the latest Firmware and Firmware Updater Tool.

Disconnect all devices from the PC running DOTS.



Extract the .zip file containing the MPS Firmware Updater. In the extracted folder, double-click on the MPS Firmware Updater Application to start the Firmware Updater Tool.



Stop the DOTS Software backend, if it is running on your computer. You can do so with a right-click on the DOTS tray icon and a subsequent click on “Stop backend” (Adminisitrator privileges required).

Click on the button and select the Firmware file (.dotsfw) from its current location on your computer.

Connect a single MPS device via USB to your computer. The Firmware update starts as soon as the device is recognized, and the update progress is visualized on the bottom of the Tool.

Figure 26: DOTS Firmware Updater Tool.

Firmware Update Troubleshooting

Problem	Solution
The Firmware Update does not start “COM port access denied” or “Device search failed”	Reconnect the MPS and restart the update process. The update process must be started first, only then connect the MPS (within 20 sec after starting the update process).
The Firmware Updater tool stops before 100 % update progress.	End the Firmware Updater via the Windows Task Manager (Ctrl+Shift+Esc). If an error message appears, close it. The MPS needs to be restarted manually before a new update process can be started: Disconnect the MPS from the computer. Use the pin tool (same as a SIM pin tool to remove SIM card holder from a smartphone) to press the tiny button next to the USB port for > 15 seconds.

	 Restart the Firmware Updater Tool, but this time, start the updating process just right before USB-connecting the MPS.

MPS blinking patterns

The blinking patterns described below describe the MPS state and can only be observed while no measurement is taking place.

Location	Pattern	Information
Center Window	**Yellow** continuous for < 1 sec, then blinking and all LEDs light up once	Sensor is initializing after reset. Sensors are automatically switched on when USB-connected to a PC.
Center window	**Green** every 5 sec	Sensor heartbeat. The sensor is switched on, but idle (no measurement running at that moment).
Center window	**Red** continuous Blinking every ~ 2 sec Blinking faster than every 2 sec	USB connected, charging. Battery close to being empty. Battery ~ 50 %. Battery close to being full.
Center window	**Blue** continuous	Sensor will switch off due to lack of DOTS Software connection in 5 seconds, 15 minutes after disconnecting the device from USB.

These blinking patterns can be observed during measurements.

Location	Pattern	Information
Round window (DO module)	**Red** flashing	DO measurement in progress.
Center Window	**Various colors** flashing or continuous light	Backscatter or Fluorescence measurement in progress. The LED color and blinking pattern depends on the settings. Note that the 385 nm, 850 nm, and 940 nm LEDs may not be visible by eye.

Contact

Website	www.scientificbio.com	
Newsletter subscription	https://www.scientificbio.com/subscription-center Information on new Software versions, Device firmware updates, new products	
eMail	insights@scientificbio.com	
Headquarters	SBI North America	SBI Europe Represented by: aquila biolabs GmbH
	201 Penn Center Blvd Pittsburgh, PA 15235	Arnold-Sommerfeld-Ring 2 52499 Baesweiler, Germany
	+1 412-230-8463	+49 2401 8049708

MPS experiment checklist

- [ ] Is the USB cable that connects the MPS Hub to the PC routed with sufficient slack within your shaker?
- [ ] Is an available MPS connected to the MPS Hub (Connected sensors are visible in Device list, Devices appear as connected)?
- [ ] Is the shake flask filled with uncooled, noncondensing cultivation medium?
- [ ] Is the medium inoculated without leaving any tips or toothpick inside the flask?
- [ ] Have you added antifoam to your culture in applications with baffled shake flasks?
- [ ] Is the shake flask mounted in a way that no labels or labeling fields are located above the MPS sensor windows?
- [ ] Is the shaker darkened/covered for fluorescence applications with increased sensitivity requirements?
- [ ] Is the DO Sensor Pill (if added to flask) circulating well inside the shake flask at the desired rpm?
- [ ] Have you generated, documented, and started a new experiment in DOTS Software?