



Omega Series

O P E R A T I N G M A N U A L

Revision K

Omega Family of Readers

The **FLUOstar Omega** is a multifunctional microplate reader that supports a wide variety of applications and the following reading modes: fluorescence intensity, time-resolved fluorescence, absorbance and luminescence.

The **POLARstar Omega** can measure in the same modes as the FLUOstar, as well as in fluorescence polarization mode with simultaneous dual emission.

The **LUMIstar Omega** is a luminescence microplate reader that can be upgraded to include all of the above mentioned modes.

The **SPECTROstar Omega** is an absorbance microplate reader that can be upgraded to include all of the above mentioned modes.

The versatile optical system allows easy switching from top to bottom optic.

All Omega instruments achieve high-performance measurement data in a wide range of wavelengths. Additionally, the instruments have a built-in incubator and can be configured with up to two reagent injectors.

This manual is designed to guide FLUOstar Omega, POLARstar Omega, LUMIstar Omega and SPECTROstar Omega users through the basic hardware features of the instrument.

Although these instructions were carefully written and checked, we cannot accept responsibility for problems encountered when using this manual. Suggestions for improving this manual will be gratefully accepted.

BMG LABTECH reserves the right to change or update this manual at any time. The Revision Number is stated at the bottom of every page.

Manufacturer: BMG LABTECH GmbH
Allmendgrün 8
77799 Ortenberg
Germany
www.bmglabtech.com
techsupport@bmglabtech.com
+49-781-969680

For more contact information please visit www.bmglabtech.com or send an email to germany@bmglabtech.com.

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TABLE OF CONTENTS

<u>1</u>	<u>Technical Specifications</u>	<u>4</u>	<u>6</u>	<u>Description of Components</u>	<u>11</u>
<u>2</u>	<u>Safety Information</u>	<u>6</u>	6.1	Optics	11
2.1	Description of Warnings	6	6.2	Installation and Changing of Optics	11
2.2	General Information	6	6.2.1	Fluorescence Intensity Optics	11
2.3	Environmental Safety Standards	6	6.2.2	Luminescence Optics	11
2.4	Electrical Safety	6	6.2.3	Fluorescence Polarization Optics	12
2.5	Photobiological Safety	6	6.2.4	Dual Emission Optics	12
2.6	Chemical and Biological Safety	7	6.2.5	Combination Optics	12
2.7	Disposal and Instrument Takeback/Recycling Program	7	6.2.6	Absorbance Optics	13
2.7.1	Disposal of Transport Packing	7	6.2.7	High Density Optics	13
2.7.2	WEEE Compliance	7	6.2.8	Bottom Optics	13
2.8	Cleaning and Instrument Disinfection	8	6.2.9	Advanced Optic Head for TRF / TR-FRET	13
<u>3</u>	<u>Intended Use</u>	<u>9</u>	6.3	Filters	15
<u>4</u>	<u>Installation</u>	<u>9</u>	6.3.1	Filter Change and Installation	15
4.1	Transport Lock	9	6.3.2	Fluorescence Filters	16
4.2	Software Installation	10	6.3.3	Fluorescence Polarization Filters	16
4.3	Power and Communication Connections	10	6.3.4	Dual Emission Filters	16
<u>5</u>	<u>Instrument Overview</u>	<u>10</u>	6.3.5	Absorbance Filters	16
			6.3.6	Luminescence Filters	17
			6.4	Spacers	17
			6.5	Reagent Injectors	17
			6.6	Fan Cover	18
			6.6.1	Incubation of unsealed Microplates	18

1 Technical Specifications

FLUOstar Omega

- Fluorescence intensity
- Time-resolved fluorescence – TR-FRET (optional)
- Luminescence (optional)
- UV/VIS absorbance spectra or filter based absorbance (optional)
- AlphaScreen®/AlphaLISA® (optional)
- Upgradeable to POLARstar Omega

LUMIstar Omega

- Luminescence
- Simultaneous dual emission luminescence (optional)
- Upgradeable to FLUOstar/POLARstar Omega

POLARstar Omega

- Fluorescence polarization
- Fluorescence intensity
- Simultaneous dual emission fluorescence
- Time-resolved fluorescence – TR-FRET (optional)
- Luminescence (optional)
- Simultaneous dual emission luminescence (optional)
- UV/VIS absorbance spectra or filter based absorbance (optional)
- AlphaScreen®/AlphaLISA® (optional)

SPECTROstar Omega

- UV/VIS absorbance spectra
- Upgradeable to FLUOstar/POLARstar Omega

Light Source

- FLUOstar, POLARstar & SPECTROstar Omega: High-energy xenon flash-lamp

Detector

- Side window photomultiplier tube
- CCD with absorbance spectrometer

Filters

- FLUOstar & POLARstar Omega: 2 filter wheels with 8 excitation and 8 emission filter positions
- LUMIstar Omega: Emission filter wheel with 8 filter positions

Gain Control

- Software selectable gain
- Automatic gain adjustment

Plate Carrier

- Auto lock microplate carrier
- All microplate formats up to 1536-well
Microplates should fulfill the SBS specification and non-SBS formats should fit: (l × w × h) max.: 128 mm × 86 mm × 22 mm; min. length: 124 mm

Reagent Injection

- Up to two built-in reagent injectors
- Individual injection volumes for each well
- Injection volumes definable down to 3µl
- Up to 4 independent injection actions per well
- Variable injection speed (100 µl/s to 420 µl/s)

Shaking

- Linear, orbital and double-orbital shaking
- User-definable time and speed

Incubation

- Incubation range from ambient +4°C to 45°C, in 0.1°C steps
- Extended incubation up to 65°C (optional)
- Temperature monitoring (without incubation)
- Temperature stability 0.2°C

Fluorescence Intensity

- Limit of detection < 10pM (< 0.2 fmol/well fluorescein, 384sv,20µL)
- Spectral range (ex. and em.): 240 ... 740 nm (900 nm optional)

Time-resolved Fluorescence

- Limit of detection < 30 amol/well europium
- High end TRF/TR-FRET: < 3 amol/well europium
- Spectral range (ex. and em.): 240 ... 740 nm (900 nm optional)

Luminescence

- Limit of detection < 20 amol/well ATP
- Spectral range 240 ... 740 nm

Fluorescence Polarization

- Limit of detection: <5 mP SD at 1 nM fluorescein
- Spectral range: (ex. and em.): 380 ... 740 nm (900 nm optional)

Spectrometer based Absorbance

- Spectral range 220 ... 1000 nm
- Full spectrum captured in < 1 s/well
- Selectable spectral resolution: 1 nm, 2 nm, 5 nm, 10 nm
- OD range: 0 to 4 OD
- Accuracy: < 1% at 2 OD
- Precision: < 0.5% at 1 OD and < 0.8% at 2 OD

Filter based Absorbance

- Spectral range 230 ... 740 nm (900 nm optional)
- OD range: 0 to 4 OD

AlphaScreen®

- Limit of detection: < 5pM (< 100 amol / well (384)*)
- AlphaScreen® and AlphaLISA® possible

Computer Interface

- USB 2.0 or USB 3.0

Power Requirements

- 100-240 V, 50/60 Hz, max. peak 300 VA
- Consumption:
Instrument off: 0 W
Normal operating conditions: 25 W
Normal operating conditions, incubation set to 37°C: 60 W
Fuses: T5A/250V (use original spare fuses provided by BMG LABTECH only)

Dimensions

- Height: 31 cm, width: 44 cm and length 48 cm

Weight 29 kg

Ambient Conditions

- Operating temperature: 15°C to 35°C
- Storage temperature: -10°C to 50°C
- Humidity of atmosphere: 20% to 80%
- Non-condensing

Instrument Conformity

- Over voltage category II
- Contamination class II
- Protection class I

Options

- Stacker for 50 microplates and optional barcode reader
- ACU: Atmospheric Control Unit to regulate both O₂ (1-19%) and CO₂ (0-20%) inside the microplate chamber
- Gas Vent: Passive purge gas vent to replace the atmosphere in the microplate chamber
- Fan Cover: improved control of microplate ambient conditions, especially for long- term incubation experiments
- LVis Plate with 16 low volume microspots (2µL) for e.g. DNA concentration measurements
- LVis Plate with additional absorbance quality control standards (approximate values of 0.1, 0.3, 0.6 and 1.0 OD); one holmium oxide filter for wavelength accuracy

Limit of detection was calculated according to the IUPAC standard: $3 \times (SD_{\text{blank}})/\text{slope}$

* Limit of detection < 100 amol of biotinylated and phosphorylated polypeptide (P-Tyr-100 assay kit, PerkinElmer, #6760620C), measured in white 384 small volume microplates (17 µL/well)

AlphaScreen® and AlphaLISA® are registered trademarks of PerkinElmer, Inc.

Specifications are subject to change without notice.

2 Safety Information

2.1 Description of Warnings

The warning signs used throughout this manual adhere to the description set forth in DIN 4844-2.



A general warning calls attention to a condition which is further described and must be strictly followed by the operator.



Warning for optical radiation

2.2 General Information



This instrument must be installed and used as outlined in this Operating Manual. Installation, service and any operation which requires opening of the instrument must be performed only by trained and certified personnel from BMG LABTECH. Failure to comply with these instructions will invalidate the warranty and can lead to unsafe operation of this equipment.



The area designated for the instrument should be free of dust, liquids and acidic vapor. The surface of the table should be flat and even. Avoid areas subject to vibrations and direct sunlight.



Prior to turning on the instrument the first time after installation, let the instrument adapt to room temperature for at least 3 h to avoid condensation which can cause a short circuit. BMG LABTECH will void the warranty if damage occurs to electrical and/or mechanical parts in cases where the instrument was turned on before the recommended accommodation time.



Handling and operation of the equipment must be carried out only by qualified personnel and staff trained by an official BMG LABTECH representative.



Microplates and related accessories (e.g. LVis Plate) operated at temperatures higher than 55°C must cool down below 55°C before being handled and removed from the instrument.



Samples and reagents, be it in solid, liquid, or gaseous form, must be removed from the instrument immediately after measurement to avoid corrosion and accumulation of hazardous substances inside the instrument.

Please, use sealed or lidded plates for long termed experiments as far as possible.

If not applicable keep the microplate for a time period as short as possible inside the instrument and remove it immediately after measurement.

This can be set in the Reader Control Software:

Menu: 'Settings | Program Configuration | Test Run Settings | Move plate out after finishing a test run'.

2.3 Environmental Safety Standards

The environmental safety standards for operation under norm IEC 61010-1 are met under the following conditions:

- Indoor use (adhere to the Occupational Exposure Limit Values for ECM, UPS, vibration, and sunlight when setting up the instrument in the laboratory) Altitude (up to 2000 m)
- Temperature (+15°C to +35°C)
- Relative Humidity (Maximum 80% at 31°C non condensing then decreasing linearly to 50% at 40°C)
- Mains supply voltage fluctuation (+/- 10%)
- Overvoltage category (II) acc. to IEC 60364-4-443
- Pollution degree (2) acc. to IEC 61010-1

2.4 Electrical Safety

- Connect the unit only to a grounded supply socket. The instrument is class 1 construction and must be grounded.
- Connect the unit only to a power supply with a designated voltage rating corresponding to the label on the back of the instrument.

2.5 Photobiological Safety

This instrument is rated in the free group as RG 0, according to the IEC 62471-2 photobiological safety of lamps and lamp systems. The intended and routine use of this equipment and its high power xenon flash lamp does not pose a photobiological hazard, given that the user strictly follows these precautions:



Do not operate the instrument unless the instrument shell is mounted and all screws are in place and tightened.

Do not open the flap door of the microplate carrier during a measurement run.

Do not open the reagent door during a measurement run.

Do not disconnect a light guide during a measurement run.

Do not look directly into a disconnected light guide during a measurement run.

A measurement run is indicated by a blinking purple LED on the instrument.

The following light source is incorporated and encapsulated in the BMG LABTECH 'Omega' microplate reader instrumentation:



Xenon high power flash lamp – emits ultraviolet light during operation at levels that could injure the eyes or skin. Do not look directly at the light and avoid exposing any skin to the light. During normal operation the user will not be exposed to any light from the xenon flash lamp.

2.6 Chemical and Biological Safety

Daily routine use with this instrument may involve the handling and use of compounds that are toxic, flammable, or biologically harmful. When working with materials and compounds as stated, make sure to observe the following precautions:

- Handle all samples, be it liquid, solid, or in gaseous form according to good laboratory practice.
- Adhere to the maximum workplace concentration (MAC) and to laboratory safety regulations (e.g. BGI 850-0, formerly BGR120 in Germany).
- Wear safety goggles as the spilling of liquids may occur.
- Contact your safety officer to dispose of hazardous waste solutions and when working with flammable liquids. The microplate reader must not be exposed for any length of time to chlorinated hydrocarbons at high concentration (i.e., chloroform, aromatic hydrocarbons, such as toluene or benzene, etc.) or to acetone. All warranties are void if the instrument comes in contact with organic solvents.

The exposition of the reader to following substances must be strictly avoided. All warranties are void if the instrument comes in prolonged contact with these substances:

- Dimethylsulfoxide (DMSO), especially vapors are extremely corrosive
- Acyl Halides (e.g. acetyl chloride)
- Polychlorinated biphenyls (e.g. Arochlor 1248)
- Aromatic hydro carbonates (e.g. phenol)
- Halogenated hydro carbonates (e.g. chloroform)
- High concentrated inorganic acids prone to evaporation (e.g. HCl, HNO₃)

Organic solvents (e.g. acetone, toluene, benzene etc)

If using any of these substances while performing a measurement, be sure to remove the sample immediately after measurement is completed. If evaporation of any of these substances occurs inside the instrument, it is important to vent the instrument for a sufficient amount of time by opening the lid and placing the microplate carrier in the plate out position with the flap door open.



Gas vent connection – for the standard gas purge vent use a regulator followed by a flow restrictor to set the flow rate. The instrument should be housed in an atmospheric gas tight bag (e.g. Aldrich® AtmosBag, Z530220-1E) to prevent spillage of gas.



Use only mild detergent or 70% ethanol for cleaning the instrument. Make sure the instrument is always in the OFF position for cleaning and servicing.

2.7 Disposal and Instrument Takeback / Recycling Program

We strongly recommend storing the transport box in case you need to ship the instrument back for upgrade or repair. In case of disposing the unit please adhere to these guidelines:

2.7.1 Disposal of Transport Packing

Straps to fix the packing on pallet, edge protection, air cushion styrofoam, and plastic foil must be disposed of and recycled according to your local “plastic recycling” policy.

Transport cardboard box must be disposed of and recycled according to your local “paper recycling” policy.

2.7.2 WEEE Compliance

The European Union Waste Electrical and Electronic Equipment Directive (WEEE) is a Producer Responsibility Directive aimed at reducing the waste from electrical equipment, increasing recovery and recycling rates of WEEE and improving environmental performance of all operators involved in the life cycle of electrical and electronic equipment.

The directive specifies that, as of August 13, 2005, manufacturers of specified electronic equipment will be obliged to take back these products at the end of their useful life.

BMG LABTECH will comply with the WEEE take back requirements for BMG LABTECH branded product sold in the EU after August 13, 2005.

BMG LABTECH customers based in Europe can take advantage of the BMG LABTECH Takeback and Recycle Program. Please check BMG LABTECH's website for country-specific availability.

This Takeback and Recycle Program ensures that you can ship the product back to BMG LABTECH for proper handling at the end of its useful life. Disposal will be carried out in an environmentally safe manner using processes that comply with all current eWaste regulations where applicable.

Upon completion of the recycling process BMG LABTECH will provide, on request, a Certificate of Destruction (COD). This will release your business from any further liability for the equipment.

The program is accessible via the web at <http://www.bmglabtech.com/en/about-us/legal/weee/>

2.8 Cleaning and Instrument Disinfection

Please follow all instructions carefully for a successful disinfection of this instrument.

All parts of the instrument, that have the possibility of contacting patient sera or positive samples, have to be handled as if they are hazardous. For this reason, it is recommended that gloves be worn while maintaining or working with the instrument.

It is very important that the instrument is thoroughly disinfected before maintenance or before removing the instrument from the laboratory. Be sure that the instrument is disinfected before you send it to your distributor or to the manufacturer. For safety reasons, you have to fill out the Disinfection Certificate, or the instrument may not be accepted by the service center or by customs authorities.

Use suitable disinfectants, e.g. Alcohol (70%).

Authorized personnel wearing disposable gloves and protective clothing should only perform the disinfection procedure. The location should be well ventilated.

Disinfection Steps

1. Disconnect the instrument from the main power supply.
2. Remove the USB cable from the connector.
3. Clean all outside surfaces of the instrument carefully with cotton wool, which has been soaked in disinfecting solution.
4. Place the instrument in a large plastic bag along with the cotton wool that has been soaked in disinfecting solution. Ensure that the wool does not touch the instrument.
5. Close and seal the bag.
6. Keep the instrument in the plastic bag for at least 24 hours.
7. After the disinfection time has lapsed, remove the instrument from the plastic bag and clean all outside surfaces of the instrument with cotton wool that has been soaked in alcohol solution.
8. Repeat the procedure for disinfection on any accessories, which will be returned with the instrument.
9. Complete the Disinfection Certificate

DISINFECTION CERTIFICATION

This instrument and its inventory have never been in contact with any dangerous biological material, or if so, the instrument and its inventory have been disinfected according to the instructions of the operating manual of the instrument.

Name: _____

Company: _____

Date, Signature:

3 Intended Use

The OMEGA multi-mode microplate reader microplate reader is intended for professional laboratory research use by trained personal who understand the nature of fluorometry, photometry and luminometry.

The instrument may be used only for research and development or other non-clinical purposes.

For validation of the entire system it is recommended that Good Laboratory Practices (GLP) are followed to ensure reliable analyses.

4 Installation

When moving the Omega reader, it is recommended that two people lift the instrument together to avoid personal injury. Be sure to lift the instrument from the bottom and both to avoid injury and/or damage to the instrument. The operator of the Omega microplate reader is assumed to be trained in the correct operation of the instrument and the safety issues.

When unpacking the instrument, please check to ensure that all of the following parts are included:

- FLUOstar Omega or LUMIstar Omega or POLARstar Omega or SPECTROstar Omega reader
- Control and MARS Data Analysis software
- Manual
- Power cord
- USB cable
- Service box containing:
 - Allen key (1.5mm)
 - 2 extra fuses: 5A/250V for main power 100V- 240V
 - 4 spacers
 - Injector needle cleaner (only with instruments that have reagent injectors)

Call BMG LABTECH immediately if any of these items are missing.

The area designated for the instrument should be free of dust, liquids and acidic vapours. The table's surface should be flat and even. Avoid areas subject to vibrations and direct sunlight.

A minimum of 10 centimeters of open space is required behind the instrument. Space above the instrument is required to access the reagent door.

When moving the Omega reader, it is recommended that two people lift the instrument together to avoid personal injury. Lift the instrument from the bottom and both sides.

After unpacking and positioning the reader, please do the following steps in the given order:

1. Release transport lock
2. Install software
3. Plug in power and USB connection

4.1 Transport Lock

When the instrument is shipped or moved to a different location, the transport lock should be in the locked position.

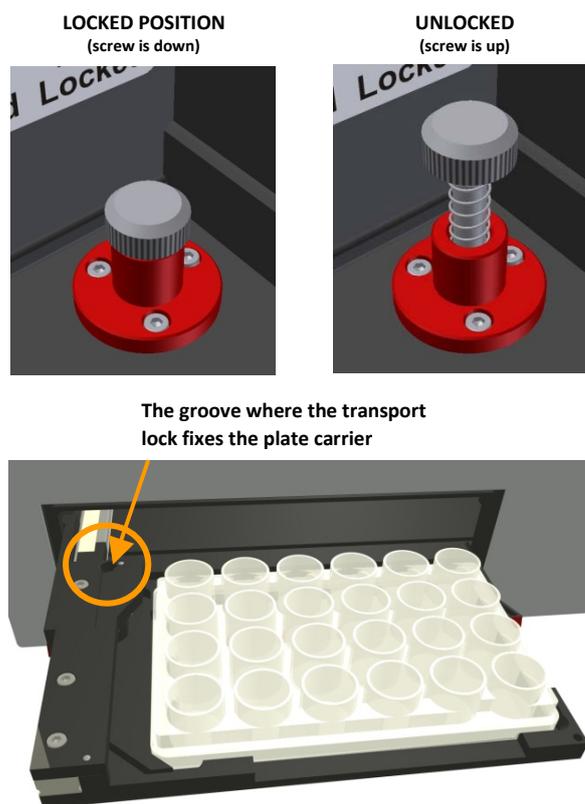


Figure 1: Top left: transport lock in locked position.
Top right: transport lock in unlocked position.
Bottom: the groove where the transport pin can lock the plate carrier

The transport lock is located in the back left corner of the reagent box (figure 1 and figure 6). Once the instrument is in its permanent location, the transport pin should be unlocked to free the plate carrier. To do this turn the transport pin counter-clockwise until it is moved up by the spring.



If the instrument needs to be moved to a new location, the plate carrier should be in the locked position otherwise the transport system could be damaged.



Figure 2: Plate In / Plate Out button

Press and hold the plate in / plate out button for 3 seconds, hereafter the plate carrier will automatically move to its lock position. Once the reader is switched off, the transport pin can be moved down and turned clockwise. The transport pin must be screwed until it tightens. Please tighten it firmly with your fingers. Don't use any tools.

The transport system is locked when the transport lock is in its down position and firmly tightened.

4.2 Software Installation

! Before connecting the instrument's USB communication cable the Control Software must be installed!

Follow the instructions given in the software manual for both installing the Reader Control and MARS Data Analysis software.

4.3 Power and Communication Connections

Power Connection

First check that the power switch on the back of the instrument is in the 'Off' position. Inspect the voltage information on the label next to the power switch to ensure that it corresponds to the local main power specifications. Also make sure the power cable is grounded. Hereafter, the power cable can be connected to the instrument.

USB Communication Connection

Connect the USB cable to the FLUOstar Omega (or POLARstar Omega or SPECTROstar or LUMIstar Omega) and to the USB port on the PC. Please connect the reader directly to your PC and do not use a USB-hub.

! Only connect a computer that corresponds to EN 60950 and UL 1950 for data processing instruments.

You can perform a connection check within the setup menu of the Omega software (go to 'Settings | Connection' and click 'Connection check').

If the instrument and PC are communicating, a 'Connection OK' message will appear.

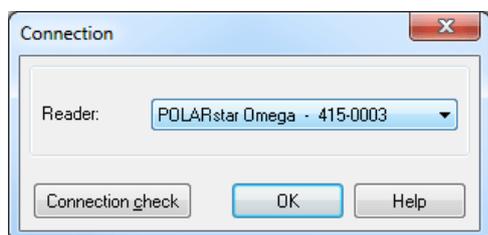


Figure 3: Connection window

5 Instrument Overview

Front View

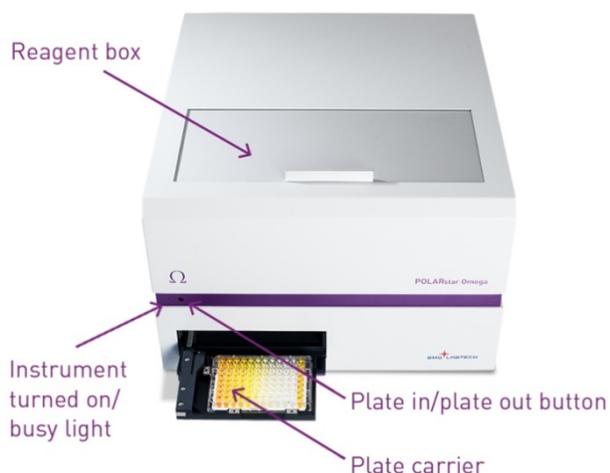


Figure 4: FLUOstar Omega

A constant green light means the instrument is turned on. A flashing green light means the instrument is busy (e.g. performing a measurement, plate in/out, priming, etc.). A faster flashing green light (5 flashes per second) means an error has occurred.

Back View

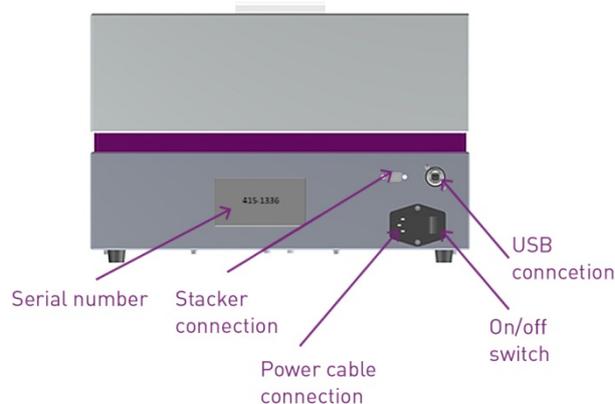


Figure 5: Back view of reader

Top View, Reagent Box

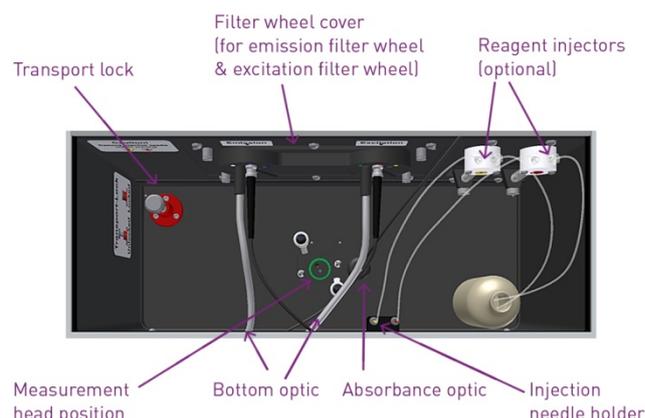


Figure 6: FLUOstar Omega & POLARstar Omega top view of reagent box with spectrometer based absorbance and 2 reagent injectors; no top optic is installed

6 Description of Components

6.1 Optics

All readers are equipped with standard UV/Vis optics for top reading. For the Omega, there are different top reading optics available for fluorescence intensity, fluorescence polarization, luminescence, absorbance and time-resolved fluorescence.

6.2 Installation and Changing of Optics

The Omega readers are equipped with quick-fix mountings (figure 7), allowing for the easy exchange of optics and placement of position spacers (for more about spacers see 6.4). No tools are required for changing the optics. Remove the optic from the positioning wheel (figure 8) by hand and turn the holders on the measurement head frame to release the black mounting piece of the optic.

Quick-fix: in released position



Quick-fix: in fixed position

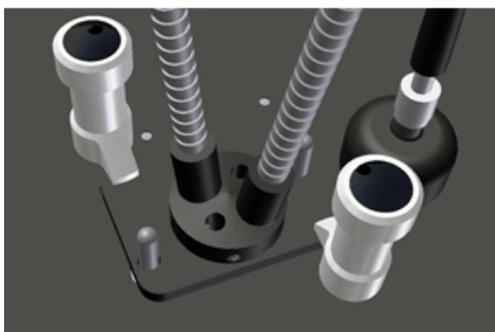


Figure 7: Quick-fix - pull up and turn to change optic

Always hold the optics by the black mounting piece!

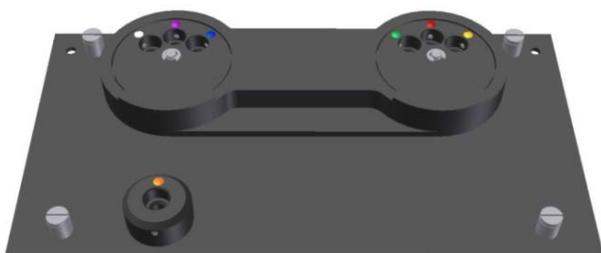


Figure 8: Filter wheel cover with positioning wheels

6.2.1 Fluorescence Intensity Optics

The fluorescence intensity and time-resolved fluorescence light guides are silver in color, liquid-filled and come connected to the excitation and emission positioning wheels. The yellow marked light guide routes the excitation light to the sample while the blue marked light guide routes the emission light to the detector.

In addition, the Fluorescence Intensity Optic can be used to measure luminescence. To do so the emission light guide is used to route the light from the sample to the detector. The luminescence option is of course needed for reading luminescence.

If the AlphaScreen option is present, this optic can be used to detect AlphaScreen.

To position the measurement head with the holders, see chapter 6.2 *Installation and Changing of Optics*.

If the reagent injection option is present, be careful when positioning the needles in the measurement head (see figure 10) in order to avoid damage to the reagent needles and to the optic.



Figure 9: Fluorescence intensity optic

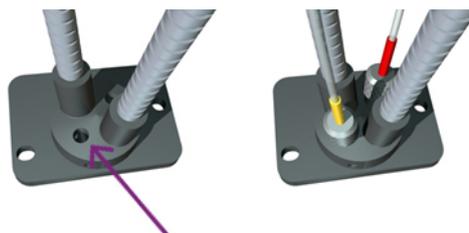


Figure 10: Holes to position reagent needles in the measurement head

6.2.2 Luminescence Optics

The Luminescence Optics are important for LUMIstar Omegas, but can also be used in FLUOstar or POLARstar Omegas. The luminescence optic has one light guide, which is silver in color. The light guide connects to the emission side. To position the measurement head with the holders, see chapter 6.2 *Installation and Changing of Optics*.

There are two dedicated luminescence optics: a 3 mm diameter light guide that is used for plates up to 96-wells, and a 2 mm diameter light guide that is used for plates up to 384-wells.

For reading luminescence, the luminescence option needs to be present in the FLUOstar or POLARstar Omega.



Figure 11: Luminescence optic

6.2.3 Fluorescence Polarization Optics

The light guides for fluorescence polarization are black and form a triangle at the base of the optic.

Position the light guides as follows: right light guide into excitation position, left light guide into the upper emission position (PMT 1) and the center light guide into the lower emission position (PMT 2).

Only the POLARstar Omega can measure fluorescence polarization. It is possible to upgrade the FLUOstar Omega to a POLARstar Omega.

Regarding filters for fluorescence polarization see chapter 6.3.3.



Figure 12:
Fluorescence polarization optic

6.2.4 Dual Emission Optics

The dual emission optics are designed for assays in which you excite at one wavelength and measure two emission wavelengths simultaneously (i.e. FRET). This is only possible in the POLARstar Omega because this action requires two PMT's. The dual emission optics look like polarization optics, but are not capable of polarization. The optic has to be installed in the same way as the polarization optic (figure 12).

6.2.5 Combination Optics

This optic can only be used with readers with PMT based absorbance, not with spectrometer based absorbance.

The combination optic is made up of two liquid-filled light guides for fluorescence intensity or luminescence and a quartz fiber for absorbance measurement (figure 13). It can be used for plate formats up to 96-well.

To position the measurement head with the quick-fix holders, see chapter 6.2 *Installation and Changing of Optics*.

For fluorescence measurements: excitation enters through the yellow marked light guide and emission is measured through the blue marked light guide.

For absorbance measurements: The gray, red marked, absorbance light guide excites from above and the absorbance is measured through the bottom optic.

In addition, for reader which have the luminescence option available, luminescence can be measured through the blue marked light guide.



Figure 13: Combination optic

Regarding reagent injection: be careful when you position the needles in the measurement head (see figure 14) to avoid damage to the reagent needles as well as to the optic. The optional reagent injectors need to be available.

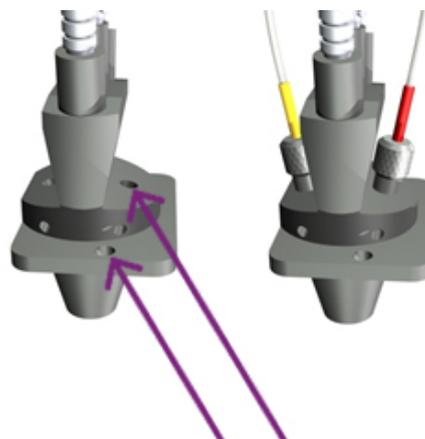


Figure 14: Holes to position reagent needles in the measurement head

6.2.6 Absorbance Optics

This optic can only be used with readers with PMT based absorbance, not with spectrometer based absorbance.

There is an optimized optic for absorbance mode available (see figure 15). This optimized absorbance optic can be used for plate formats up to 384-well. The light guide connects to the red-marked excitation-positioning wheel. This optic does not allow for the use of injectors.

You can also use the combination optic (see chapter 6.2.5).



Figure 15: Optimized absorbance optic

6.2.7 High Density Optics

The high-density optic has one excitation light guide surrounded by six emission light guides. This optic is designed to minimize cross-talk in plate formats such as 384- and 1536-well plates.

To position the measurement head with the quick-fix holders, see chapter 6.2 *Installation and Changing of Optics*.

The single black light guide connects to the yellow-marked position of the excitation wheel. The six-bundled light guides are inserted into the blue-marked position of the emission wheel.

To measure 1536-well plates the reader has to be prepared specially.

Injection is not possible into 1536 well plates.



Figure 16: High density optic

6.2.8 Bottom Optics

The bottom optics are used to measure fluorescence, luminescence and absorbance. The bottom optics enters the reagent box on the left side and are connected to the left position of the excitation and emission wheels.



Figure 17: Principle drawing of top and bottom optics

6.2.9 Advanced Optic Head for TRF / TR-FRET

The Advanced Time-Resolved-Fluorescence (TRF) – Optic Head is an upgrade option for the FLUOstar Omega and POLARstar Omega microplate readers. It is based on an innovative optical system using a lens and z-adjustment to direct the excitation light to the optimal focal point in the center of a well.

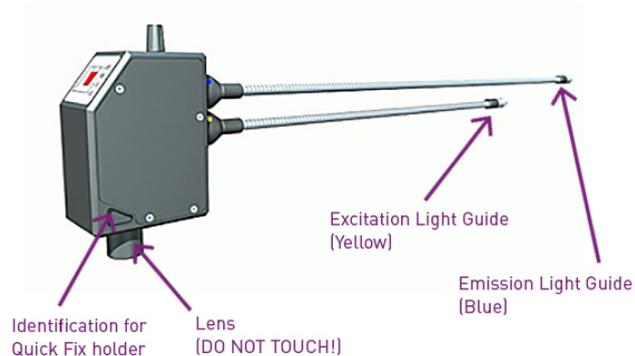


Figure 18: The TRF- optic head with excitation and emission light guides

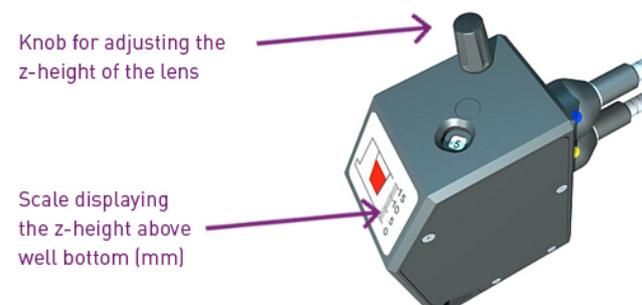


Figure 19: The TRF- optic head with z-height adjustment

Installation:

Notes: Before installing the TRF-optic head make sure to install the supplied filters for TRF measurement as described in 6.3.1 'Filter Change and Installation'.

Make sure that the firmware of your Omega reader is at least v.1.12. The firmware version can be read by opening the Control Software and clicking on the reader icon in the upper right corner. Contact BMG LABTECH in case you need an update of the reader firmware (support@bmg-labtech.com).

1) Open the lid of the reagent box of the FLUOstar/POLARstar Omega and remove top optic if installed. Take the body of the TRF-optic head in the left hand, the light guides in your right hand and feed them through underneath the already installed excitation light guides. The light guides should be carefully placed outside of the instrument to prevent them from scratching the inside of the reagent box. (Figure 20).

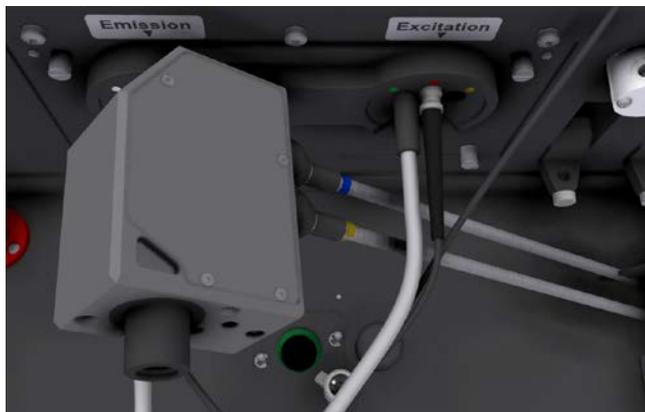


Figure 20: Feeding the light guides of the TRF-optic head

2) Hold the Advanced TRF-optic head vertically inside the instrument. Turn it 45 degrees and position it so that the lens is perpendicular towards the open hole of the measurement position. Carefully lower the TRF optic head and feed the head through the two rising bars pointing upwards from the bottom. Now the head should rest smoothly on the bottom of the reagent box.



Figure 21: Turn the TRF optic head 45 degrees

3) Turn the lower right Quick-fix clockwise until it touches the base of the TRF optic head. Hold the optic head with your left hand while lifting up the Quick-fix with your right hand approx. 7 mm and turn further clockwise until the lever touches the wall.

Release the Quick-fix and it will snap-on and hold the optic head in position. Repeat this procedure for the upper left Quick-fix (Fig. 22).



Fig 22: Schematic of the Quick-Fix holder (left) and actual image of the TRF-optic head in the instrument (right).

Connect the excitation light guide (yellow) to the matching pin hole (yellow) of the Excitation Positioning Wheel.

Be careful NOT TO OBSTRUCT the black light guide of the absorbance spectrometer. The excitation light guide of the TRF optic head must reside BELOW the black light guide of the absorbance spectrometer (Fig. 23).

Connect the emission light guide (blue) to the matching pin hole (blue) of the Emission Positioning Wheel.



Fig. 23: Final look of the installed TRF optic head and light guides. Note: The excitation light guide must be positioned BELOW the black thin light guide of the absorbance spectrometer!

Use of the Advanced TRF Optic Head:

The best sensitivity of a fluorescence measurement in a microplate well can be achieved by exciting the fluophore at the z position of the maximal light absorption. This is done by adhering to the following z-heights when standard 96 or 384 well plates and volumes are used (Fig. 24).

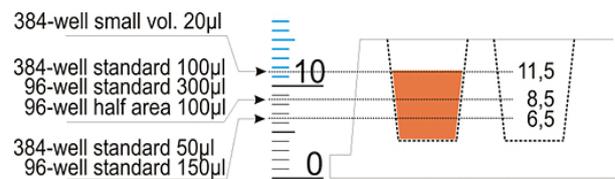


Figure 24: Recommended z-height positions for respective well formats and volumes

- 1) Use the knob located at the top of the TRF-optic and set the z-height according to the plate format and well volume.
- 2) Dispense a known volume of your assay with the highest concentration in well B2. Insert the microplate into the reader.
- 3) Start the Reader Control software, go to 'Settings | Program Configuration | Additional Options' and select 'Automatic open current state display'.

Next, click on the Test Protocol tab, select the button for 'Time Resolved Fluorescence' and double click on 'TRF_OpticHead_Adjust' to open the protocol.
- 5) Make sure to select the correct microplate and the correct excitation and emission filters.
- 6) Double check the layout to make sure well B2 is selected. If you have dispensed your assay solution into a different well, change the layout accordingly.
- 7) Set the Gain to 2300 (for white microplates) or 2500 (for black microplates). In general, BMG LABTECH recommends white plates and 200 flashes for best performance.
- 8) Start the protocol and double-click on the well currently being measured. A window appears and displays the TRF raw data (RFU) / time.
- 9) Now, turn the knob on the Advanced TRF-optic and watch how the intensity is changing. Move the lens up and down in 1 mm steps after approx. 10 sec to see 4-5 data points / setting.

The following image shows how to determine the optimal z-height for maximal intensity:

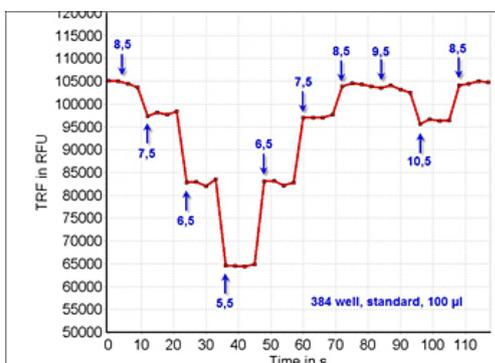


Figure 25: Determination of the best z-height in a 384 well plate format. Note the change in intensity at different z-heights. The optimal z-height was set at 8,5 mm.

6.3 Filters

In the FLUOstar Omega, 4 excitation and 4 emission filters are factory installed; in the POLARstar Omega, 4 excitation and 5 emission filters are factory installed. (Filter selection varies with instrument configuration. If your unit is equipped with luminescence, then a lens will be installed. The PMT based absorbance option will also add filters.)

The position of the factory-installed filters can be found in the shipment information that accompanies the instrument.

After installation of the software, the filters must be typed in the filter table (figure 26). In the Control software, the filter table can be reached by choosing 'Settings | Filter' (see the software manual for additional information).

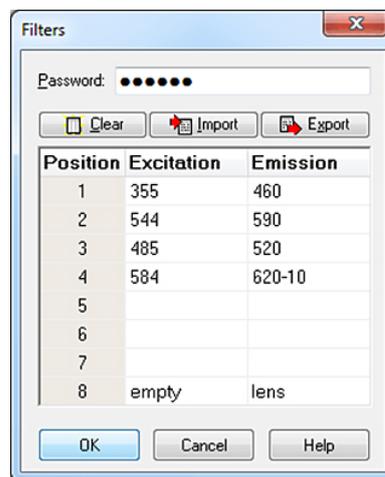


Figure 26: Filter table with example of entered values

6.3.1 Filter Change and Installation

All BMG filters have an arrow printed on the side to indicate the direction in which they should be installed. The arrow should point in the same direction as the light. If the filter is being installed in the excitation wheel, the arrow should point outwards towards the front of the unit. If the filter is to be placed in the emission wheel, the arrow should face inward towards the rear of the unit.

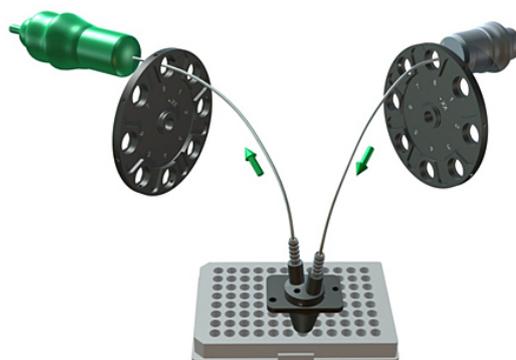


Figure 27: Direction of the light

The excitation filters and the emission filters are located in their respective filter wheels behind the filter wheel cover (figure 28 to figure 30). To access the filters, first remove the light guides. The filter wheel cover can then be removed by loosening the 4 thumbscrews (figure 29).



Figure 28: Filter change / filter installation: First remove the optic (valid for all readers)



Figure 29: Filter change / filter installation: Loosen the 4 screws

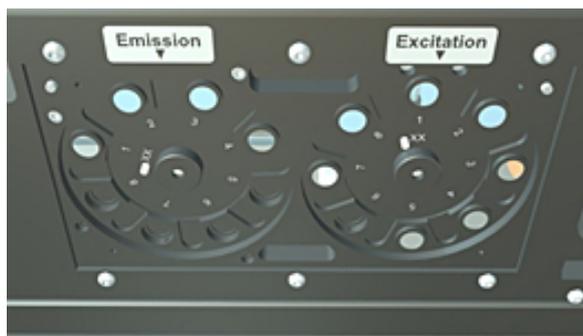


Figure 30: Filter change / filter installation: Loosen the filter wheel by loosening the nut in the middle (see text for detailed description).

The filter wheel itself (figure 31) can be removed by loosening the large retaining nut in the center (figure 30). Put a finger on the filter wheel (careful not to touch any filters) and turn the nut counter clockwise. The filter wheel can be pulled straight out of the housing.

The filter positions are numbered 1 to 8. When installing new filters note the positions and enter the information in the filter table in the Control software.

Once the filter is in place, it should be fixed with the Allen key and a small filter screw. Place the small screw in the hole on the side of the wheel and turn it only until it is snug (not too tight).



Figure 31: Filter wheel

Note: Do not place a screw in a position that is not equipped with a filter; the screw may slowly loosen and fall out and cause damage to the filter wheel mechanism.

Next to the axle in the center of the housing is a small positioning pin. This pin must fit into one of the holes on the back of the filter wheel. Replace the filter wheel on the axle and push it into position (the axle should stick out 3mm). Turning the filter wheel quickly might help position it correctly. Place the filter wheel nut on the axle and tighten it with your fingers until it is fixed. Spin the wheel again. The filter wheel should move freely and without vibration. If the wheel seems to drag or wobble, tighten the nut further, or remove the nut and reposition the wheel.

Replace the cover and reconnect the light guides.



If the instrument makes a grinding sound it is very likely that the large filter wheel nut in the middle should be tightened further, or that a filter screw is loose.

6.3.2 Fluorescence Filters

The fluorescence filters have a bandwidth that varies depending upon the type of fluorescence filter. Filters optimized for specific fluorophores can vary in the bandwidth from around 10 nm to around 30 to 40 nm (measured at ½ height). BMG-10 filters are symmetrical filters that in ½ height have a bandwidth of 10 nm and hence can be used in both the excitation and the emission position.

6.3.3 Fluorescence Polarization Filters

The excitation filters can go in any available position in the excitation wheel. The emission wheel has two filters of the same wavelength for each channel (one for parallel and one for perpendicular). They must be positioned 180° from each other (for example position 3 and position 7 for the 520 filter). The filter configuration can be entered in 'Settings | Filters'.

6.3.4 Dual Emission Filters

As for fluorescence polarization, the excitation filter can be placed in any position on the filter wheel. The emission filters to be used in dual emission measurement must be positioned 180° from each other (emission position 1 pairs with position 5, position 2 pairs with position 6, etc.).

6.3.5 Absorbance Filters

Absorbance filters are only needed in readers that use PMT based absorbance. Place the filter (either a BMG-10 or an 'Abs' filter) that matches the wavelength of choice in any excitation position. The light goes through the 'excitation filter' into the absorbance optic. It then passes through the sample and is collected in the bottom optic. Hereafter the light passes through the 'emission filter' wheel to the detector. Depending on your reader options, the following settings must be used:

Reader with Luminescence option:

A lens is mounted into emission filter position 8. This lens can also be used for the 'emission path' of the absorbance measurement. Please type 'lens' into emission position 8 of the filter table.

Reader without Luminescence option:

Define a filter position (typically position 8) as empty so that all the light from the reaction will pass the emission filter wheel directly to the detector ('empty' needs to be typed into the filter table and the corresponding emission filter wheel position must be empty).

Use of an additional filter:

You may choose to use an additional second filter beside the one in the excitation filter wheel to block any stray light. The filter can be used in any emission filter position and must be selected properly in the protocol definition.

6.3.6 Luminescence Filters

Like for fluorescence filters, the luminescence filters are designed to transmit as much light as possible. The bandwidth varies depending upon the type of assay (filters are optimized to return the best result) and can vary in bandwidth from around 10 nm to around 100 nm (measured at ½ height).

6.4 Spacers

The Omega readers are designed for most microplate formats. The height of some microplates exceeds the space allowed under the optic. The minimum space between the optic and microplate should be 1.5 mm. With 6, 24, 48-well plate formats, it will be necessary to raise the optic using the spacers provided in the service box.

The spacers are metal rectangular pieces with a hole in the center. Each spacer is 2 mm in height. They are installed between the measurement head and the bottom of the reagent box. The number of spacers used depends on how high the optic needs to be elevated.

Determination of the number of spacers:

If the height of the microplate exceeds the height of the left border of the plate carrier, (see figure 32) spacers need to be installed under the measurement head (see figure 33). There should be enough spacers so that the height of the left side of the plate carrier is slightly higher than the microplate.

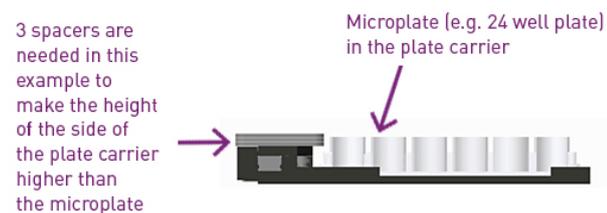


Figure 32: Front view of the plate carrier

Installation of spacers:

If you install spacers, first remove the injection needles (if any) from the optic and then remove the optic.

Install the appropriate number of spacers, using the positioning pins as a guide. Reinsert the optic.

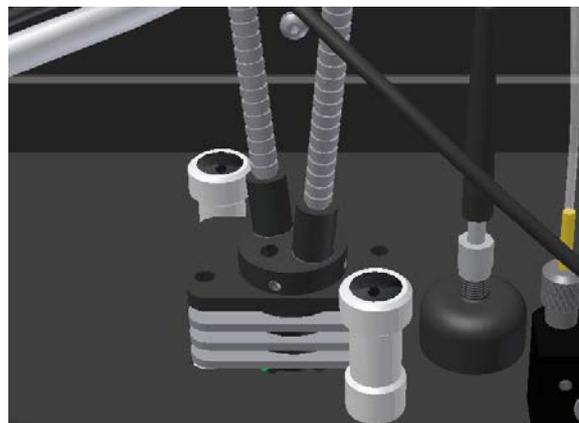


Figure 33: Example of spacers between measurement head and bottom of reagent box

As a cross check (to ensure that the microplate can pass under the optic), push the plate carrier manually into the instrument and slowly move it towards the optic. There should now be approximately 1.5 to 2 mm of space between the optic and microplate.

6.5 Reagent Injectors

The Omega readers can be equipped with up to 2 reagent injectors (figure 34).



Figure 34: Reagent injectors

When the reagent injector(s) are not in use, the needle(s) can be placed in the needle holder (figure 6).

The reagent needles are made of stainless steel, the tubings and valve housing are made of Teflon and Kel-F, and the syringe barrel is made of glass. All reagent injector materials are among the most chemical resistant materials that are available.

The needle tip plays a major role regarding the pumps' accuracy. Always treat the needles with care. That is, be careful when positioning the needles in the measurement head or in the needle holder.

For obtaining optimal performance of the reagent injectors, please see the following chapter.

Note: Before using a pump in a test run, it is necessary to prime the pump. This can be done using the Control software (menu command 'Measure | Prime'). It is also possible to prime the pumps without using the software. After opening the reagent door, locate the two buttons on the left side. To prime a pump double click the respective button.

Use and Maintenance of the Reagent Injectors

To remove cellular debris and viscous solutions from the syringe barrel:

Take off the syringe barrel and rinse it with distilled water. It may be useful to use the wire syringe cleaners (found in the service box) to scrape particles off the walls.

In order to obtain optimal performance from the reagent injectors, it is recommended to follow these guidelines in the use of the reagent injectors:

- Do not use the syringes more than two cycles without liquid.
- After each use, thoroughly flush the syringes with distilled water.
- If the plunger is removed from the syringe barrel, it should be wiped with ethanol before replacing.
- Syringes should be cleaned each week using one of the following procedures:

Cleaning with weak detergent or 10% bleach

1. Fill the syringe with a weak detergent or 10% bleach solution
2. Leave the solution in the syringe for 30 minutes
3. Flush the syringe a minimum of 10 times with distilled water

Cleaning with acid / base (best procedure if cells are used in the syringe)

1. Fill the syringe with 0.1M NaOH and leave in the syringe for 10 minutes.
2. Flush the syringe a minimum of 10 times with distilled water.
3. Fill the syringe with 0.1M HCl, and leave the solution in the syringe for 10 minutes.
4. Flush the syringe a minimum of 10 times with distilled water

6.6 Fan Cover

The Fan Cover is optional. It allows soaking ambient air into the instrument. The airflow is from the front to the back of the reader. The fan ventilating capability has the following advantages:

- to ventilate the reader after long test runs at higher temperatures to remove excessive humidity in the reader and to avoid corrosion
- to reach desired temperatures for long-term incubation experiments more quickly
- to stabilize the environmental conditions during a measurement close to ambient conditions

The 'Fan Control' mode in the Omega readers is only accessible if firmware version 1.30 or higher is installed and the reader is equipped with a Fan Cover.

Please see the various possible settings in the Software Manual Omega Part II under Settings – Fan Control.

6.6.1 Incubation of unsealed Microplates

Frequent long time incubation of open microplates can accumulate excessive humidity inside the instrument and can be avoided by using the optional fan cover.

Incubating for short time is possible without fan cover by respecting:

- Reader control software: →Settings →Program Configuration
 - Plate-Out after Reading
- Venting by opening the Instrument Reagent Box Slider while the Microplate Carrier is out. Please do not change incubation temperature. The necessary venting time is similar to the incubation time before.