

OLYMPUS®

INSTRUCTIONS

scan[®] AUTOMATED IMAGE ACQUISITION HARDWARE

This instruction manual describes the hardware components controlled by the Olympus **scan**[®] Automated Image Acquisition Software. To ensure safety, obtain optimum performance and familiarize yourself fully with the use of these products, we recommend that you study this manual thoroughly before operation. Together with this manual, please also read the **scan**[®] Automated Image Acquisition Software manual as well as the manuals of all other devices controlled by this software in order to understand general operation methods. Retain this manual in an easily accessible place near a system for future reference.

OLYMPUS SOFT IMAGING SOLUTIONS GMBH

Rupert-Mayer-Strasse 44

D-81379 München

Tel: +49 89 - 89 55 805 660

Fax +49 89 - 89 55 805 6606

Email: info@olympus-sis.com

www.olympus-sis.com

Imaging Excellence

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CE Declaration of Conformity

OLYMPUS BIOSYSTEMS GMBH

Robert-Koch-Strasse 9
D-82152 Planegg

declares under its own responsibility that the following products

cell^R

Imaging Station for Life Science Applications *

to which this declaration refers are in conformity with the

Low Voltage Directive 73/23/EEC

and the

EMC Directives 89/336/EEC and 93/68/EEC

and the following standards:

EN 61010-1: 2001 (Low Voltage)

EN 61326:1997 + A1: 1998 + A2: 2001 (EMC).

Michael Czempiel and Dr. Matthias Seel, Managing Directors

October 08, 2005

* The hardware of the basic scan^R system is identical to that of the basic cell^R system.

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1 Introduction and Overview

Thank you very much for purchasing Olympus' state of art Screening Station for Life Science **scan[®]** and for your confidence in our products and services. It is Olympus main objective to provide you with solutions able to meet your experimental demands and thus pave the way to your scientific success.

The hardware described here constitutes essential parts of the Olympus Screening Station **scan[®]**. The hardware components are intended to be controlled via the **scan[®]** Acquisition Software for automated image acquisition of biomedical samples. In combination with the **scan[®]** Acquisition Software the hardware is intended for the use in biomedical research.



The **scan[®]** Analysis Software, the **scan[®]** Acquisition Software as well as the hardware components of the Olympus **scan[®]** Screening Station for Life Sciences are for research use only.

1.1 Abstract

The Screening Station for Life Science scan[^]R is based on the hardware of the cell[^]R Imaging Station and consists of the following hardware components:

- Illumination System MT20
- Illumination Coupling to the microscope (epi-fluorescence illuminator with light fiber)
- Real-time Controller board inside PC
- CCD camera

You will find a detailed description of these components, handling and alignment instructions as well as advice how to maintain the performance of your system on the following pages. Keep in mind that all the components are designed to work together as an integrated system. Taking away or replacing single components without prior consultation of Olympus Soft Imaging Solutions is strongly discouraged and will most probably impair the performance of the system dramatically. Any damages to the system due to such mishandling will not be covered by the guarantee.

All necessary tools for handling and alignment are shipped with the system.

The Imaging Station scan[^]R is compatible with the Olympus microscope IX81.

An additional installation of a standard Olympus epi-fluorescence Xe, Xe/Hg or Hg lamp housing e.g., U-LH100HGAPO, U-LH75XEAP0) is not possible because the microscope's fluorescence excitation port is occupied by the OBS epi-fluorescence illuminator.

This instructions manual is for the hardware components of the Olympus Soft Imaging Solutions Screening Station scan[^]R. To ensure safety and optimum performance as well as to familiarize yourself with the use of the system we recommend you to study this manual thoroughly before operating the system. Keep this manual in an easily accessible place near the Imaging Station for future reference.

The scan[^]R system meets the CE, UL and FCC requirements.

IEC/EN 61010-1(2nd ed.), UL61010-1,







EN61326 Class A, FCC Part 15 Class A (Illumination System MT20: class A and B).

1.2 Technical Support

If you find any information missing in this manual or you need additional support, please contact your local Olympus representative.

1.3 Symbols

The following symbols are placed on the Illumination System MT20. Study the meaning of the symbols and always use the equipment in the safest possible manner.

General symbols	
Symbol	Explanation
	Carefully read the instructions on the label and the manual before use. Improper use could result in personal injury to the user and/or damage the equipment.
	The surface becomes hot and should not be touched with bare hands
	Parts may be under high voltage and should not be touched.
	The main switch is turned OFF.
	The main switch is turned ON.
	This symbol indicates separate collection of waste electrical and electronic equipment in the EU countries. Do not throw the equipment into the domestic refuse Please use the return and collection systems available in your country for the disposal of this product

1.4 Checklist: Standard scan[^]R Components

Before assembly, please check whether you have the complete set of standard scan[^]R system components as listed below.

- a** Microscope IX81 with control unit IX2-UCB
- b** Illumination System MT20 consisting of:
 - MT20 housing with integrated lamp house, filter wheel, shutter and attenuator
 - short arc burner
 - power cord
 - Illumination System connector cord (blue RJ45 cable)
 - Light sensor connector cord (see photo in Chapter 7, System Assembly and Adjustment)
 - optical fiber (quartz light guide; see photo in Chapter 7, System Assembly and Adjustment)
 - set of hex-wrenches
 - cotton gloves
- c** Fluorescence filter set consisting of:
 - Dichroic mirror and emission filter mounted in Olympus filter cube
 - Excitation filter(s) to be mounted in the MT20 filter wheel
- d** MT20 epi-fluorescence illuminator to couple the illumination system to the microscope via the optical fiber
- e** CCD camera
 - FireWire™ (IEEE 1394) cable
 - trigger cable
- f** Motorized stage with controller
- g** cell[^]R imaging computer including
 - cell[^]R Real-time Controller board
 - FireWire™ (IEEE 1394) card
 - Front panel with three digital I/O ports
 - scan[^]R acquisition and analysis software installed
 - computer monitor with cable
 - 2 power cords
 - software protection key (dongle)
- h** scan[^]R acquisition and analysis software on CD-ROM
- i** Manuals
- j** Z-drift control nit ZDC mounted on the IX81 frame (optional)
- k** Climate chamber (optional)

2 Illumination System MT20

This chapter refers only to the fluorescence Illumination System MT20. Before using this unit together with the other system components, the microscope and associated devices, make sure that you have carefully read and understood their respective manuals, and understand how the entire system should be operated.

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2.1 Safety Precautions and Handling Instructions



For Laboratory Use Only.

Danger: Ultraviolet radiation is emitted from this product. Avoid exposure. ALWAYS WEAR PROTECTIVE CLOTHING. EXPOSURE MAY CAUSE PREMATURE AGING OF THE SKIN AND CANCER. ALWAYS WEAR PROTECTIVE EYEWEAR; FAILURE TO DO SO MAY RESULT IN SEVERE BURNS OR LONG TERM INJURE TO THE EYE. Never look directly into the lamp. Exposure can cause eye and skin allergy and allergic reactions. Medications or cosmetics may increase your sensitivity to ultraviolet radiation. Consult a physician before operating this product if you are using medications or have a history of skin problems or believe yourself particularly sensitive to sunlight.

Always have the microscope's UV cut plate mounted as protection against UV radiation.



Color fading of specimens: This system features high excitation light intensity to ensure bright observation of dim fluorescent specimens. Consequently, after longer periods of observations using high-power objectives, the colors of specimens will fade quicker than usual, causing the intensity and contrast of fluorescent images to deteriorate. In such cases it is advisable to reduce excitation light intensity to slow down color fading. To reduce the light intensity use the built-in attenuator as explained in this manual.

Engage the shutter of MT20 as explained in this manual (or use the shutter of the microscope) even if you interrupt observation only for a short time.

2.1.1 MT20 Safety Precautions

- a Always use a power cord provided or approved by Olympus Soft Imaging Solutions.
- b Provide unimpaired access to the main power switch at the rear panel.
- c No electrical cord should be connected or disconnected while the power switch is set on ("I"). Make sure that the unit's main power switch is set off ("O") before plugging or unplugging the power cord to the power outlet.
- d Always make sure that the **grounding terminal** of the Illumination System MT20 and the wall outlet are connected properly. If the System is not grounded, Olympus Soft Imaging Solutions cannot warrant the electrical safety and performance of the system.
- e When installing the Illumination System MT20 leave at least 100 mm free air space at the rear panel and at least 50 mm at the sides and on top to allow air circulation. The Illumination System MT20 has an internal fan to cool the arc burner. The air outlet is located at the rear of the housing while the inlet grid is at the bottom.

- f** The Illumination System MT20 must be placed on an even, stable and flat surface and standing on its supports. Make sure that no cloth or paper can clog the air inlet slits at the bottom of the housing.
- g** The arc burner has to be exclusively one of the two types specified by Olympus Soft Imaging Solutions. No other arc burner may be used with the Illumination System MT20.
- h** Do not open the burner flap of the Illumination System MT20 while the arc burner is turned on. First turn off the arc burner by software and wait for at least 30 minutes while the cooling fan is operating. Set the main power switch on “**O**” (OFF) before opening the burner flap. The arc burner socket and all parts in the vicinity of the burner will be extremely hot during operation and will cause thermal injury if touched.
- i** Never attempt to disassemble the Illumination System MT20 or open its housing. The internal power supply units and electrical circuits contain high voltage components and can cause severe electrical shock and thus are not serviceable by the user. Warranty will be lost if the housing is opened.
- j** Do not operate the Illumination System MT20 under environmental conditions other than specified in this manual (see *Chapter 2.2.6., Specifications and Technical Data*).
- k** Do not look directly into the light emitted by the arc burner with unprotected eyes under any circumstances. The emitted light is very intense and has a very high UV-content which may cause irreversible damage to your eyes.
- l** Avoid direct exposure of unprotected skin to the light emitted by the arc burner. It can cause severe burns.
- m** Direct exposure to the arc burner light may occur at the following positions:
 - Illumination System’s housing at the fiber port if the fiber is removed
 - Exit of the optical fiber
 - Exit of the illumination coupling (epi-fluorescence illuminator)
 - Beam path of the microscope (which upon proper usage is not accessible by the user)
 - Exit of the objective (or at the nose piece, if no objective is mounted)
- n** If the equipment is used not as specified by this manual, the safety and performance of the equipment may be impaired. In addition, the equipment may also be damaged and warranty may be lost. Always use the equipment as outlined in this instruction manual.

2.1.2 Special Safety Precautions Regarding the Arc Burners




- a** Handling the arc burner bulb is potentially dangerous. Wear a protective mask, leather gloves and a thick long-sleeved shirt or coat during handling and disposal (see below).
- b** The arc burner bulb is under high internal pressure. Do not hit the arc burner bulb against anything nor scratch it or apply excessive stress. Avoid vibrations. Otherwise the arc burner bulb could explode and cause injuries.
- c** For transport and storage always place the arc burner in the provided protective case.
- d** Take care to avoid leaving fingerprints or dirt on any part of the high pressure arc burners. If contaminated, clean by wiping gently with gauze. To remove fingerprints or oil stains, wipe with gauze slightly moistened with lens cleansing fluid.
Since these fluids are highly inflammable, be careful to keep them away from open fire, heat or hot surfaces and potential sources of electrical sparks, such as main switches.
- e** The arc burners have an average life time as specified in this manual (see *Chapter 2.2.6, Specifications and Technical Data*). When the arc burner hour counter (see *Chapter 4.3.2, Burner Alignment*) gives a reading higher than the specified life time, replace the burner with a new one following the instructions for replacement (see *Chapter 4.3.1, Burner Installation and Exchange*) and disposal of used burners (see below). Arc burners that are used longer than the rated service life time will have a reduced intensity and stability, are likely to fail and might explode because of the deteriorated glass condition.
- f** Arc burners not in service must always be kept in the provided protective case.
- g** Hg/Xe burner bulbs contain poisonous mercury. They have to be treated with special care. If they explode during service mercury gas might be dispersed in the environment.
- h** Hg/Xe burners must be treated as hazardous waste. Always follow national and local laws and guidelines when disposing of Hg/Xe burners. It is recommended to commission a professional waste disposal company with the disposal. Hand over the burner in its original packaging.
- i** Xe burners: It is recommended to commission a professional waste disposal company with the disposal. Hand over the burner in its original packaging. Disposal without breaking the glass part may result in burner bulb explosion. Follow the instructions below to prevent harmful repercussions.
 - Wear protective clothing.
 - Wrap the used lamp in a thick cloth.
 - Break the glass part of the lamp into pieces using a hammer.
 - Dispose of as industrial waste (except Hg/Xe burners, see above).


2.1.3 Handling Instructions and Recommendations

- a** The Illumination System MT20 is a precision instrument. Handle it with great care and avoid subjecting it to sudden or severe impact. Also connect each cable gently.
- b** The built-in ventilating fan and the built-in filter wheels may generate vibrations and sound. Therefore it is recommended not to place the Illumination System MT20 on the same table as the microscope.
- c** With exception of the burner flap and the filter flap the Illumination System MT20 may only be opened and disassembled by authorized service personal. Otherwise warranty is lost.
- d** Before using the Illumination System MT20 for the first time check that the burner is installed correctly and that all cords are connected correctly.
- e** Before opening the burner flap for replacement of the burner, turn it off by software (OBS System Configuration) and wait for at least 30 minutes while the cooling fan is operating. Then set the main power switch on “**O**” (OFF) and open the burner flap. (Do not open the burner flap of the Illumination System MT20 while the arc burner is turned on.)
- f** Do not place the unit inside a climate chamber.
- g** Do not bend the optical fiber to a diameter of less than 45 cm. The optical fiber is fragile and may brake if bent too strongly or if torsional stress is applied.
- h** Cover the ends of the optical fiber with the protection cover if they are not attached to the Illumination System MT20 or to the microscope.
- i** Always operate the Illumination System MT20 in an upright position. Never lay it on the side during operation. This could cause the lamp to burst, damage the equipment and cause injury.
- j** Before transport of the system remove the lamp and store it safely in the provided protective case.
- k** Mind that turning the arc burner on and off repeatedly will shorten the life span.
- l** To clean the surface of the Illumination System MT20, use a lint-free, dry and soft cloth. Never attempt to use organic solvents or strong detergents.
- m** Take care to avoid leaving fingerprints or dirt on the optical filters, the lenses at the ends of the light guide or the optics of the illumination coupling. If contaminated, clean by wiping gently with special optical cleansing tissue. To remove fingerprints or oil stains, wipe with special optical cleansing tissue slightly moistened with lens cleansing fluid. Use the cotton gloves when handling light filters.
* Since these fluids are highly inflammable, be careful to keep them away from open fire, heat or hot surfaces and potential sources of electrical sparks, such as main switches.

2.1.4 Safety Labels

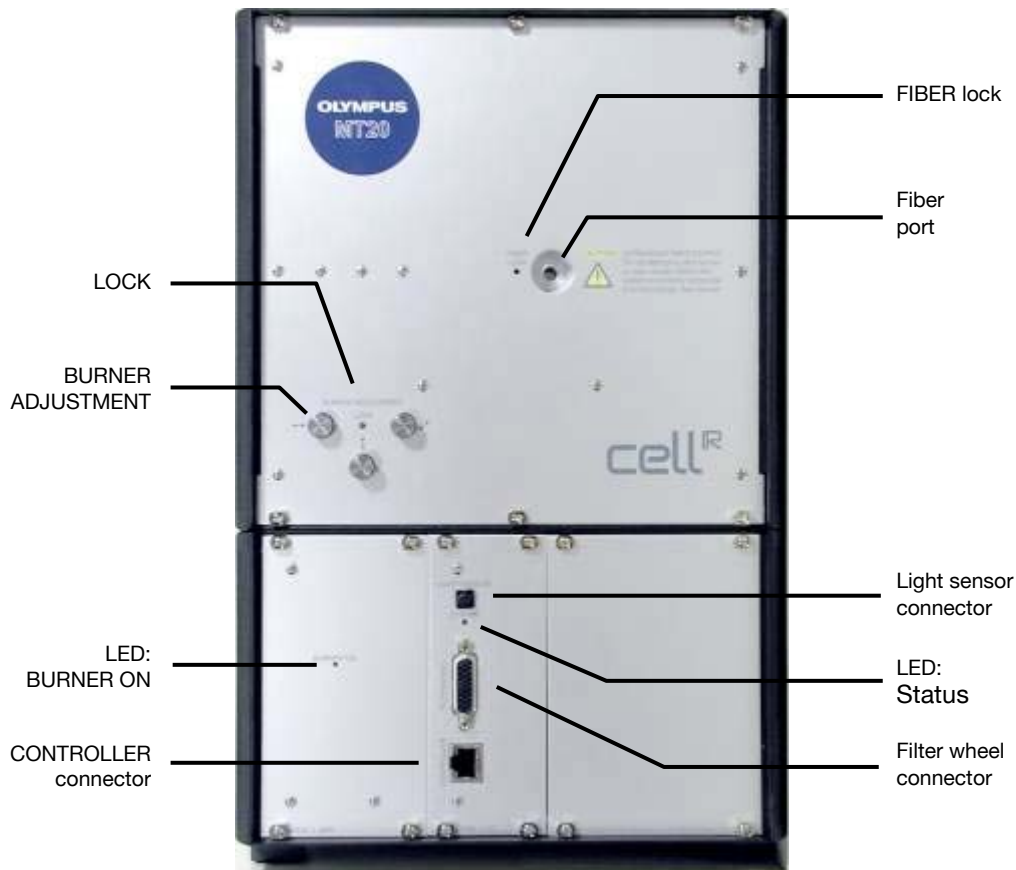
The following symbols are found on the Illumination System MT20. Study the meaning of the symbols and always use the equipment in the safest possible manner.

Lamp housing safety label		
Label	Note	Location
<p>CAUTION HIGH SURFACE TEMPERATURE INSIDE. HIGH VOLTAGE INSIDE.</p> <p>Read and understand the manual! Observe safety precautions when handling arc burners. Replace burner within the life time stated in the manual.</p> <p>Arc burner replacement:</p> <ul style="list-style-type: none"> - Turn off burner via software or manual control switch. - Wait 30 minutes for burner to cool down (ventilator). - Afterwards disconnect unit from mains. - Open burner exchange flap. - Unlock and change burner. Do not touch glass parts of the burner with bare hands. - See manual for orientation of burner tip-off and anode heat sink. Lock burner. REMOVE hex-wrench. - Close burner exchange flap properly before connecting unit to mains. - Configure burner type in software (hour counter). <p>For replacement burners and filters, please contact SALES@OLYMPUS-BIOSYSTEMS.COM</p>   	<p>Carefully read the instructions on the label and the manual before use. Improper use could result in personal injury to the user and/or damage the equipment.</p>	<p>Top side of the Illumination System MT20 / MT10</p>

Fiber port safety label		
Label	Note	Location
<p>CAUTION ULTRAVIOLET RAYS OUTPUT</p> <p>Do not attempt to start burner or open shutter before this system is correctly connected to a microscope. See manual.</p> 	<p>Carefully read the instructions on the label and the manual before use. Improper use could result in personal injury to the user and/or damage the equipment.</p>	<p>Front side of the Illumination System MT20 / MT10</p>

2.2 Illumination System MT20 Overview

2.2.1 The Front Panel



LOCK: 2 mm hex-wrench opening to lock or release the arc burner for installation and replacement.

BURNER ADJUSTMENT: three handles to align the burner.

BURNER ON LED: On / off → arc burner on / off

CONTROLLER connector: RJ45 plug to connect the Illumination System MT20 to the cell^R Real-Time Controller.

FIBER LOCK: 1.5 mm hex-wrench opening to lock or release the optical fiber in the fiber port.

Fiber port: Opening for the optical fiber.

LIGHT SENSOR connector: 3-pin connector for light sensor cable.

Filter wheel connector: 15-pin sub-D port, currently without use.

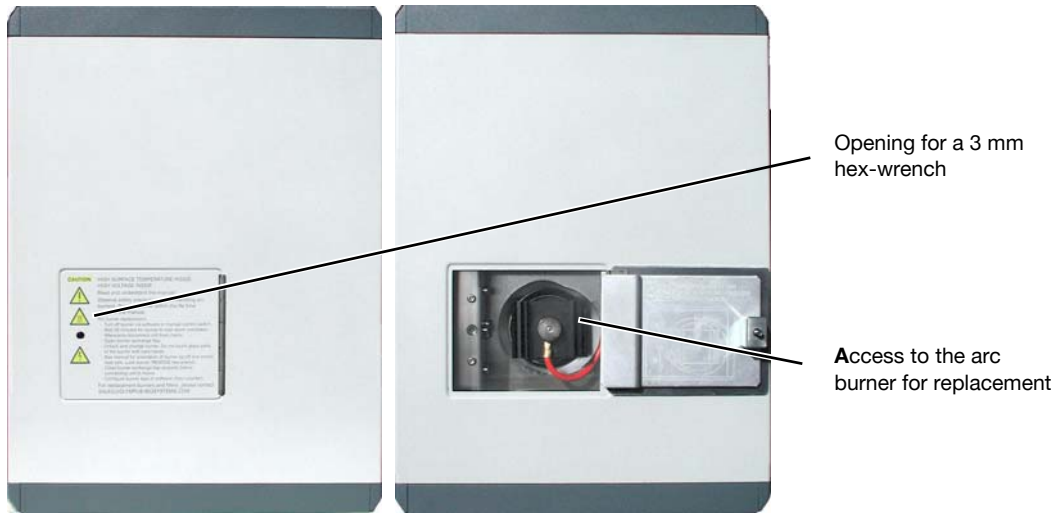
STATUS LED: Off → no connection to cell^R Real-Time Controller.

On → active connection to cell^R Real-Time Controller, ready for operation.

Regular flashing → Illumination System MT20 in stand-by mode.

Irregular activity → Illumination System MT20 in action.

2.2.2 Top: the Burner Flap



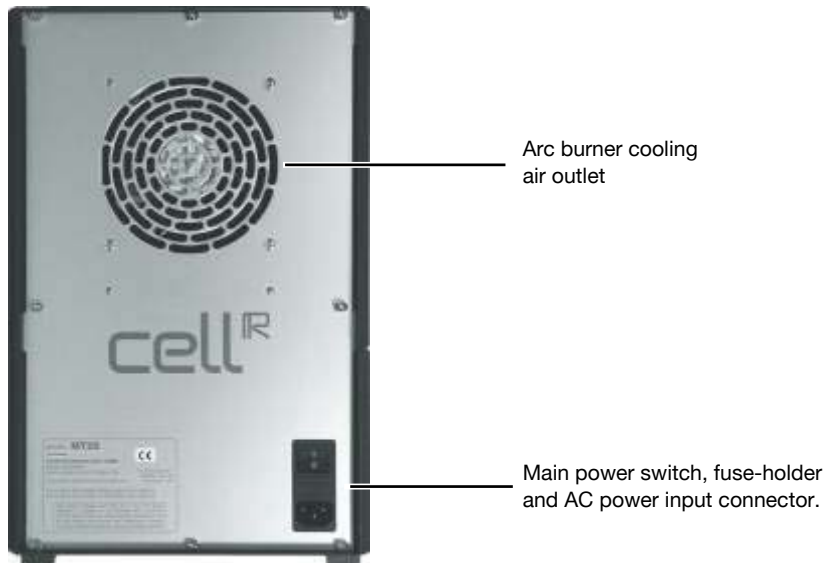
Before opening: Wait until system has cooled down, then switch main power off!

2.2.3 Right Side: the Filter Wheel Flap



The flap has to be closed and the subsequent automatic initialization has to be completed for operation.

2.2.4 The Back Panel



Do not block ventilation slits! Keep 10 cm distance from wall.

2.2.5 Operation

Only operate the system when completely installed and connected to a microscope.

For operating set main power switch on “I” (ON). By doing so the System will execute a routine to initialize and test the electro-mechanical components and motors.

For further operation run the scan^R software or the OBS System Configuration on your computer according to the software manual.

2.2.6 Specifications and Technical Data of the MT20

Dimensions and Weight: H 354 x W 236 x D 315 mm, 13 kg

Power supply rating: 100 to 120 V and 200 to 240 V AC, 50 – 60 Hz (47 – 63 Hz), 3.9 / 1.6A

Fuse: 250VAC, T4AH (5 x 20mm)

Enclosure rating: IP20

Short arc burner:

- Type 1: 150 W Xe, high pressure arc burner, Olympus _MT_ARC/XE, article No. E0435001, expected minimal lifetime under standard laboratory conditions: 1000 h
- Type 2: 150 W Hg/Xe, mixed gas arc burner, Olympus _MT_ARC/HG, article No. E0435002, expected minimal lifetime under standard laboratory conditions: 1000 h

Light output range, MT20: between 320 and 720 nm; MT20_NIR: between 380 and 800 nm

Light output power: up to 50% higher for MT20E as compared to MT20

Mechanical shutter: opening/closing time 1.0 +/- 0.2 ms

Filter wheel: 8 positions

Optical filter dimensions: Ø 24.8 mm +/- 0.1, thickness 2 – 6 mm, maximum weight 7 g each

Filter and attenuator switching time: This is dependent on the filter load and the number of positions to be moved.

Switching times [ms]						
Load [g]	< 10.5	< 21	> 21	> 28	> 35	attenuator
Next position	58	64	72	80	91	35
2 nd position	86	92	102	114	129	40
3 rd position	113	119	130	142	158	50
4 th position	141	147	157	169	186	60 etc.

Attenuator transmission [%], 14 positions: 1, 3.1, 6.3, 10, 12.5, 25, 33.3, 37.5, 50, 62.5, 66.6, 75, 87.5, 100

Operating environmental conditions: indoor use only.

- Altitude: up to 2000 m
- Ambient temperature: 5 to 40°C
- Maximum relative humidity: 80% up to 31°C, 70% at 34°C, 60% at 37°C, 50% at 40°C
- Supply voltage fluctuation: +/- 10%
- Pollution degree 2 (in accordance with IEC664)
- Over-voltage category II (in accordance with IEC664)

LED indicators, MT20 front: BURNER ON (lower left), STATUS (lower center)

Connectors, MT20 front:

- CONTROLLER: RJ45 connection to Real-Time Controller board (PC back panel)

- FILTER WHEEL: sub-D, 15-pin connection
- LIGHT SENSOR: 3 pin connection to light sensor (epi-fluorescence illuminator)

Connectors, MT20 rear:

- 3-pin AC input plug with ground
- Main switch (**I**: on, **O**: off)

Optical fiber: minimum bend diameter 45 cm short term, avoid torsional stress

- Type 1: 2 m solid quartz fiber, Olympus _MT_FIB/2m, article No. E0435007
- Type 2: 3 m solid quartz fiber, Olympus _MT_FIB/3m, article No. E0435008

2.2.7 Fuse Exchange

During standard operation the fuses will not blow. In case the fuse blows switch off the unit and contact your local Olympus service specialist or the Olympus Soft Imaging Solutions support team to avoid potential hazard because the unit might be severely damaged.

The system is equipped with 2 fuses (high breaking, delayed action fuse, 250 V AC, 4 A, size: 5 x 20 mm) located below the main switch of the Illumination System MT20 at the rear of the housing.

2.2.8 Specifications and Technical Data of the Real-Time Controller Connectors

Connectors, cell^R Imaging Computer, front, DIGITAL I/O:

- Input: $R(\text{in}) > 10 \text{ k}\Omega$, low $V < 0.8 \text{ V}$, high $V > 2.0 \text{ V}$,
- Output, low level: $R(\text{out}) < 90 \Omega$, $V < 0.8 \text{ V}$ @ $I < 5.8 \text{ mA}$
- Output, high level: $R(\text{out}) < 870 \Omega$, $V > 2.2 \text{ V}$ @ $I < 3.2 \text{ mA}$



If a high level voltage of 5 V is required, please contact your local support specialist.

Camera Trigger: open collector with 330 Ω pull-up to 5 V

- TTL high level: $R(\text{out}) = 330 \Omega$, $V > 2.0 \text{ V}$ @ $I < 9 \text{ mA}$
- TTL low level: $R(\text{out}) < 90 \Omega$, $V < 0.8 \text{ V}$ @ $I < 9 \text{ mA}$

PIFOC controller (analog out): SMB, 14 Bit DAC, $V = 0 - 10 \text{ V} (\pm 2 \text{ mV})$ @ 1 M Ω R(load), $I < 1 \text{ mA}$, $R(\text{out}) = 47 \Omega$ @ DC, suggested R(load) > 50 K Ω

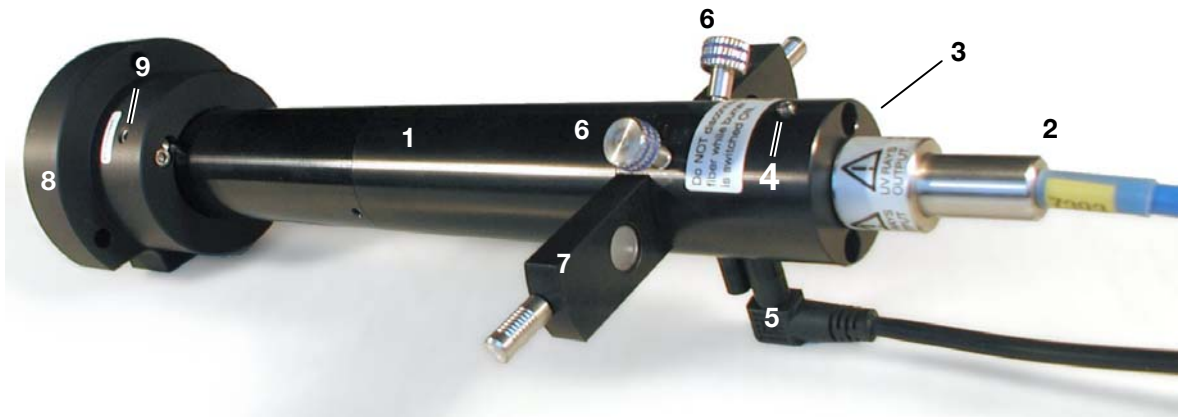
3 Epi-Fluorescence Illuminator

This chapter explains the details of the illuminator that couples the illumination system MT20 to the IX81 microscope.

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3.1 Features

The MT20 epi-fluorescence illuminator is a tube which houses the different lenses and features the fiber port, the filter slider, the adjustments screws to center the field of illumination and the light sensor to measure the light output during the optimization of the lamp position in the MT20. These items are referred to in detail below.



- | | |
|--|--|
| 1: illuminator tube containing the optical elements | 5: light sensor with cable |
| 2: light guide with fiber connector and ring to adjust depth of penetration for focus optimization | 6: adjustment screws for centering the field of illumination |
| 3: light guide port | 7: filter slider with dispersion filter |
| 4: set screw to fix the light guide | 8: flange for IX2 microscope frame |
| | 9: screw to fix the illuminator tube inside the flange |

Optionally the illuminator may have a space-saving L-shape form. Furthermore, both a straight and a L-shape illuminator containing a field stop slider is available for the IX81 microscope.

3.1.1 Light Guide Port

The light guide features a distance ring which assures the optimum position of the fiber exit when the fiber is moved all the way into the port. The position of the distance ring is set by Olympus Soft Imaging Solutions and fixed by a small screw. The fiber exit is fixed inside the port by a form-fit screw. Thus, the fiber cannot be pulled out if the fixing screw is just slightly loose.

3.1.2 Adjustment Screws for Centering the Field of Illumination

The illumination has to be centered in order to illuminate the entire chip of the CCD camera. No tool is needed to turn the screws. See *Chapter 7.4, Centering the Illumination*.

3.1.3 Filter Slider for Dispersion Filter and Exciter Balancers

The slider has four openings to hold filters of 12 mm diameter. The first position contains a dispersion filter that allows to illuminate the entire ocular's field of view. One position should always remain free for unaltered light throughput and illumination of the CCD chip with maximum brightness. The two other positions are empty by default. They can be equipped with filters which are to be fixed by easily removable plastic rings.

3.1.4 Light Sensor

A light sensor (photodiode) is mounted inside the illuminator tube (but outside the light path) which measures the intensity of back-scattered light. The more intense the scattered light, the higher the overall light intensity leaving the fiber and the better correspondingly the position of the short arc burner in the MT20 lamp house. The light sensor cable is connected to a plug in the front plate of the MT20.


3.1.5 Flange for the IX81 Frame

The illuminators are inserted into a special flange mounted on the epi-fluorescence port of the IX81 microscope; see *Chapter 4.2.2, Mounting and Connecting the Epi-Fluorescence Illuminator*.

4 System Assembly and Adjustment

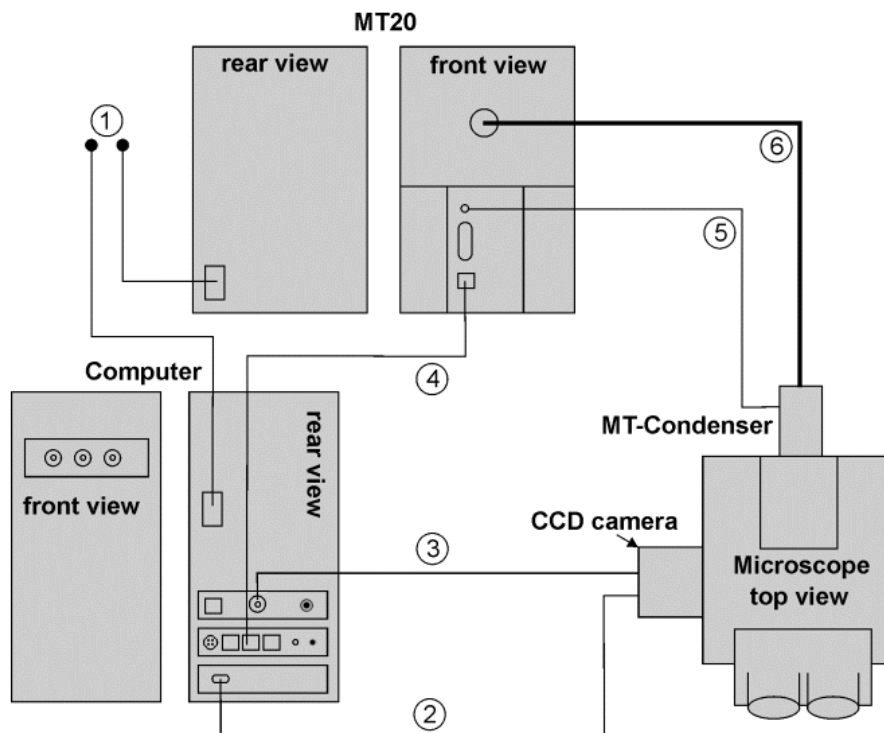
The following chapters explain how all the different hardware modules of the scan^R Screening Station have to be connected for correct operation.

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4.2	Connecting the Modules of the scan ^R Screening Station	23
4.2.1	Connecting the MT20	23
4.2.2	Mounting and Connecting the Epi-Fluorescence Illuminator	24
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 It is the user's responsibility to provide a sufficient and stable electrical power supply network for the scan[^]R system. Olympus rejects any warranty claims for any power break down follow up damage caused by an insufficient power supply network.

4.1 Overview: Connectivity of System Components

The schematic diagram below shows the connection of standard components of the cell[^]R station. Before you switch on any of the cell[^]R components, make sure that they are correctly connected.



- 1: Power cords
- 2: FireWire™ (IEEE 1394) cable to connect the CCD camera to the FireWire™ board of the PC for image data transfer.
- 3: Camera trigger line to connect the CCD camera to the computer-hosted cell[^]R Real-time Controller for synchronized image acquisition.
- 4: Illumination System MT20 connection cable: Blue RJ45 cable to connect the MT20 to the computer-hosted cell[^]R Real-time Controller / cell[^]M System Coordinator for (synchronized) control of the sample illumination.
- 5: Light sensor cable to connect the light sensor in the epi-fluorescence illuminator (to monitor the optimization of the illumination intensity) to the **LIGHT SENSOR** connector at the front of the MT20.
- 6: Optic quartz light: Blue fiber to guide the light from the MT20 to the epi-fluorescence illuminator.

 Do not bend the light guide to a radius of less than 45 cm; otherwise, the quartz fiber may become damaged.

4.2 Connecting the Modules of the scan^R Screening Station

4.2.1 Connecting the MT20

1. Connect the power cord to the main power connector at the MT20 rear panel and to a power outlet. Caution: Make sure that the MT20's main power switch is set on OFF before connection.

2. Connect the CONTROLLER connector at the front of the MT20 with the controller board at the back of the PC using the blue RJ45 cable.

3. Connect the LIGHT SENSOR connector with the light sensor in the epi-fluorescence illuminator using the black light sensor cable (with small three-pin plugs).



4. Connect the light guide: Gently insert the thin connector of the optical fiber entirely into the fiber port at the front panel. Keep the protective plastic caps for transport purposes.



The fiber is fragile and might break if handled without care.

- Do not bend the fiber more than to a diameter of 45 cm (short term: 35 cm).
- Do not expose the fiber to shear force or torque.

5. Lock the fiber by turning the fiber lock screw with a 1.5 mm hex wrench clockwise. Tighten the screw gently.

6. For the connection of the other connector of the fiber see the following chapter.



UV radiation output; do NOT disconnect fiber while burner is switched ON.

4.2.2 Mounting and Connecting the Epi-Fluorescence Illuminator

See also the photo in Chapter 3, *Epi-Fluorescence Illuminator*.



1. Insert the flange with the dovetail **1** first all the way into the frame opening.
2. Fix it with the two screws in the two holes that point downward by 45° on the left and right side of the frame using the 4 mm hex-wrench.
3. Insert the epi-fluorescence illuminator tube into the flange so that the distance between flange and back end of the illuminator (light guide port) is as listed for the different objectives.
4. Fix the illuminator with the fixing screw **2** of the flange using the 2.5 mm hex-wrench.
5. Connect the female end of the light sensor cable.
6. Insert the thick end of the light guide as far as possible (until the ring to adjust the depth of penetration makes contact with the illuminator) and fix it with the fixing screw using the 2 mm hex-wrench. The fiber cannot be pulled out if the form-fit fixing screw is just slightly loose.



UV radiation output; do NOT disconnect fiber while burner is switched ON.

4.2.3 Connecting the Camera

The camera has to be mounted on the microscope camera port using a c-mount adapter as described in the microscope manual.

4.2.3.1 Camera Hamamatsu Orca AG

1. Make sure that the camera controller unit is switched off and disconnected from the power outlet.

2. Use the 3m FireWire™ cable to connect the FireWire™ port at the back of the camera controller unit to the FireWire™ card at the back of the computer.

3. Connect the trigger-out BNC port of the controller at the back of the computer to the trigger-in BNC port of the camera controller unit.

4. Connect camera and camera controller to the special multi-pin cable.

4.2.4 Connecting the Imaging PC to the Modules

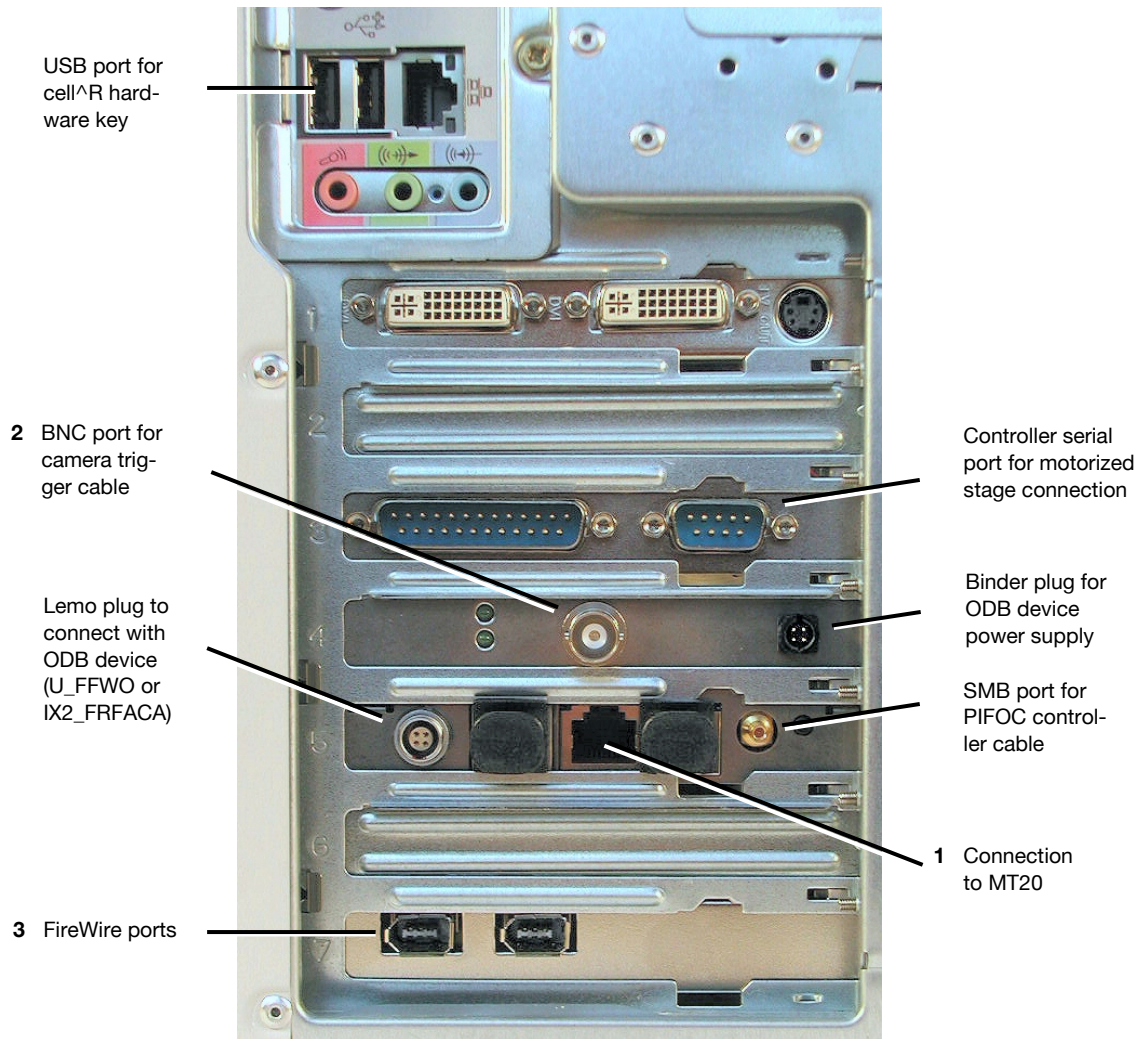
1. Insert the blue RJ45 cable coming from the **CONTROLLER** plug on the MT20 front panel into the central RJ45 port of the cell^R controller board.

2. Camera: Connect the BNC plug of the trigger cable to the BNC port of the cell^R controller board.

3. Camera: Plug the FireWire™ cable coming from the camera or the camera controller (in case of the Hamamatsu Orca AGR) into any one of the two ports of the FireWire™ card.

In addition to the interfaces at the back, the cell^R imaging computer features a **DIGITAL I/O** interface on the front panel. It contains three BNC ports (**1**, **2** and **3**) that enable to send TTL trigger pulses to peripheral devices during an experiment.





4.2.5 Configuring the Imaging PC Network Connections



When the factory settings of the network connections to the cell^R Real-time Controller are changed, the communication will be lost and the Imaging Station cannot be operated anymore.

It may happen that the settings of the network connections are changed, especially when the operating system is upgraded or the imaging PC is hooked to an intranet. The following instructions allow to reset connection settings.

1. Go to **Control Panel ▶ Network Connections**.

2. Make sure that the connection to the cell^R Real-time Controller is called CTR_R.

3. Go to **Properties ▶ General**.

4. Make sure that **Internet Protocol (TCP/IP)** is activated and go to **Properties**.

5. Use the **IP address 42.42.42.17** and the **Subnet mask 255.255.255.0**.

6. Confirm with **OK**. And go to the **Advanced** tab.

7. Make sure that the **Window Firewall ▶ Settings** are **off**.

8. Confirm with **OK**.

4.3 Preparing the Illumination System MT20

4.3.1 Burner Installation and Exchange



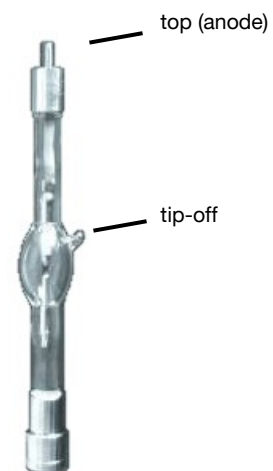
Wear protective glasses, clothes and gloves. Avoid any kind of mechanical stress to the burner. Allow the illumination system to cool down for at least 30 minutes with the main power on to keep the cooling fan in the back operating. Then set the main power switch on **O** (OFF). Do not touch glass parts of the burner.

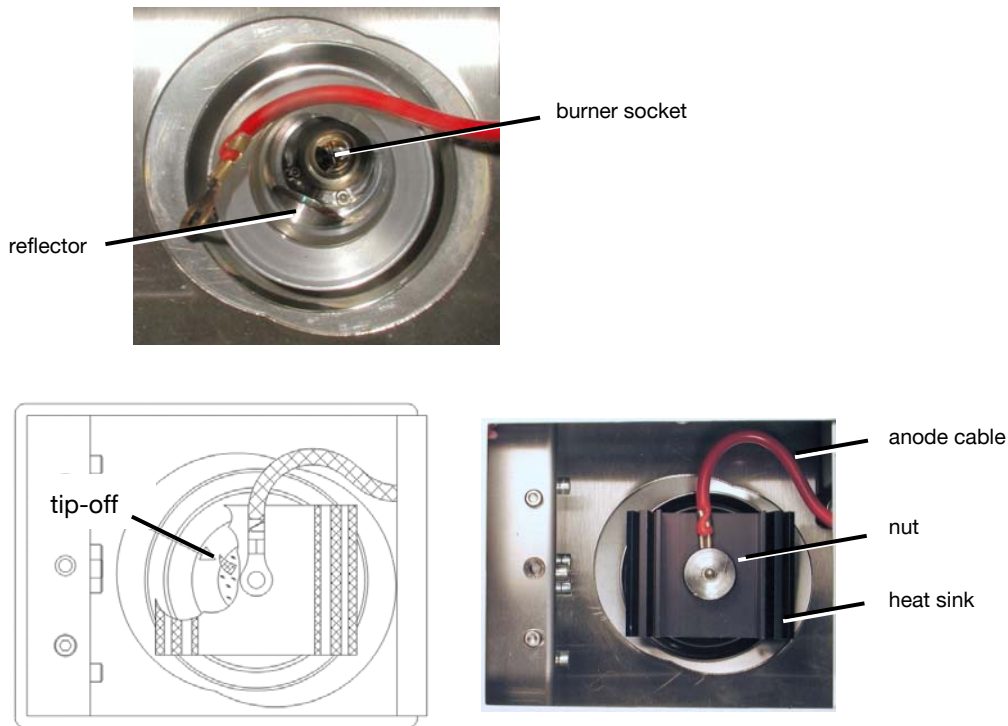
1. Loosen the burner flap screw (3mm hex wrench). The flap will lift slightly. Open the flap.

2. If a burner is installed and has to be exchanged gently hold the heat sink and unscrew the nut. Remove nut, anode cable and heat sink from the burner socket.

3. Loosen the lock screw at the front panel and take out the burner. (Storage and/or disposal see Chapter 2.1.2, *Special Safety Precautions Regarding the Arc Burners*).

4. Insert the new burner with the flat end (cathode) downwards and with the tip-off pointing in the direction indicated in the scheme engraved on the inside of the flap (see scheme below). Be sure not to scratch the surface of the reflector when inserting the burner into the funnel of a MT20E.





5. Tighten the lock screw gently but firmly until the screw is blocked; force is not necessary. The lock screw is spring loaded; you will feel the resistance of the spring once the screw is tight.

6. Attach the heat sink, the anode cable and the nut to the screw (anode) at the top of the burner. Hold the heat sink with the orientation as shown in the photo below while tightening the nut.

7. Close the burner flap and gently tighten the burner flap screw. The lamp will not ignite for safety reasons if the flap is open or just loosely closed due to an interlock mechanism.

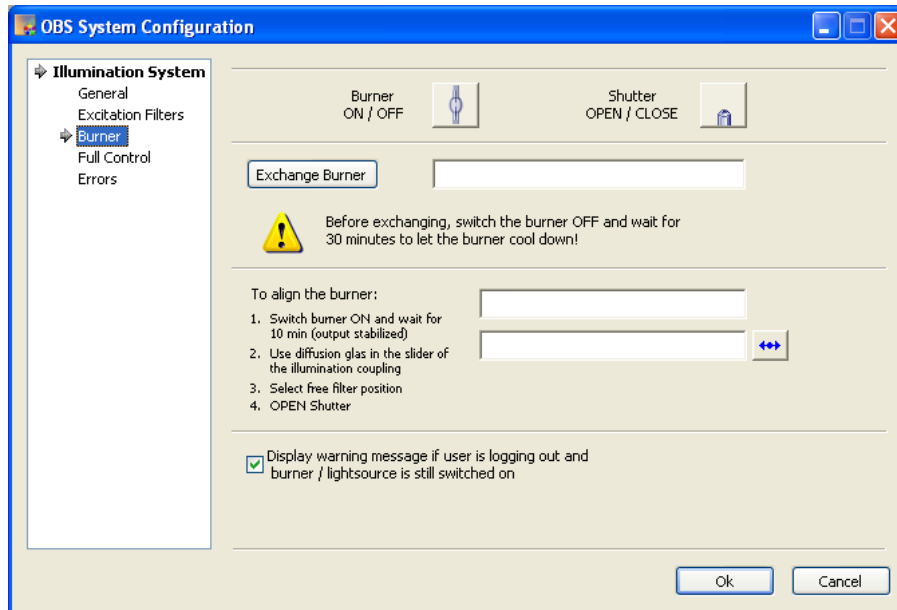
4.3.2 Burner Alignment

The burner has to be aligned after installation for maximum performance. To do so, the light output is constantly monitored by the light sensor in the epi-fluorescence illuminator.

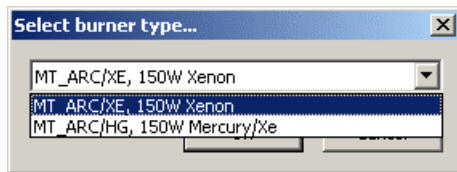


ObsConfig.exe

1. Start the **OBS System Configuration** software by clicking the **OBSConfig** button and go to the **Burner** page.



2. Check if the burner type listed is correct. If not, click the **Exchange burner** button to open the **Select burner type...** dialog box that enables you to choose the type of arc burner. Confirm by clicking **Ok**.



i If you install a different type of burner, that is, if you switch from a Xe to a Hg/Xe burner, you will be asked if the one installed is used or not. If it is new, the hour counter will start with 0 h, otherwise it will start with the last reading of this particular type of burner. Thus, if you frequently exchange the type, you will still have control of the expired lifetime of each.



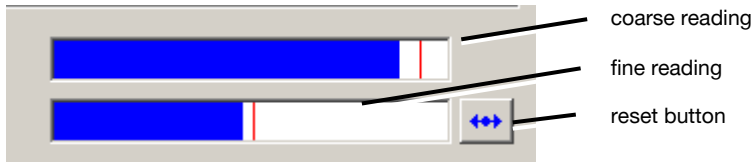
3. Start the burner by clicking on the **Burner ON/OFF** button and wait for about 10 min until the light intensity stabilizes. The light sensor in the epi-fluorescence illuminator has to be connected to the MT20 (see Chapter 4.2.1, *Connecting the MT20*).

4. Move the dispersion filter mounted in the filter slider in the back of the epi-fluorescence illuminator into the light path to increase scattered light intensity.



5. Open the shutter by clicking on the **Shutter OPEN/CLOSE** button.

6. The light output is indicated by the two blue bars. They do not represent an absolute value but are rescaled frequently. The maximum that was reached so far is indicated by the red lines.

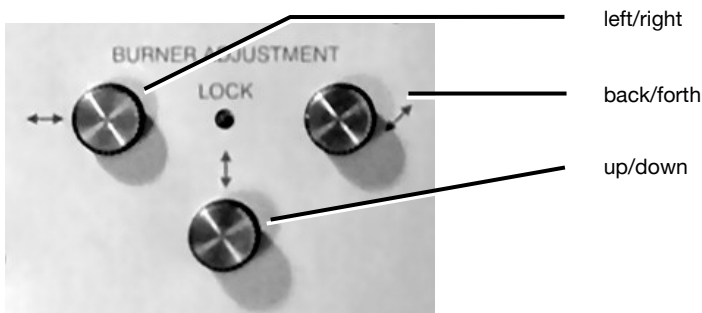


The upper bar gives a coarse reading. When the maximum reaches the right end the display is reset so that the maximum is repositioned at 80% of the length. This may happen several times during the adjustment.

The lower bar shows an area of $\pm 10\%$ around the maximum of the upper bar and therefore gives a finer reading. However, it is updated independently once its maximum reaches the right end and thus does not necessarily show a fivefold magnification of the current display of the upper bar. The reset may happen frequently.

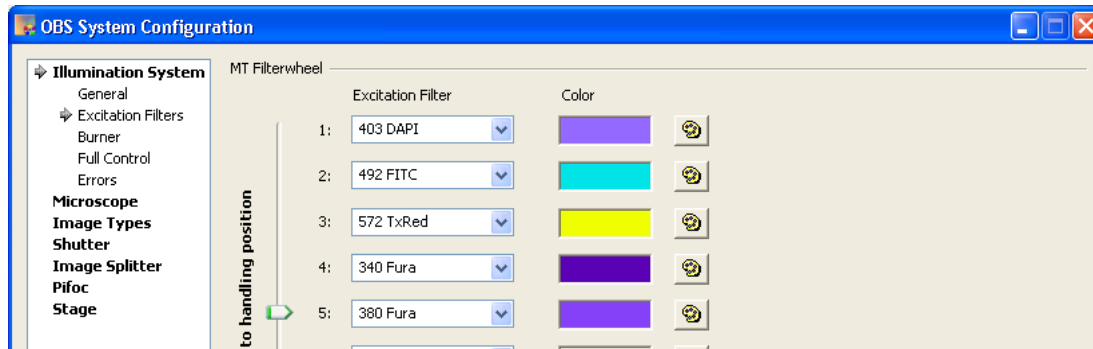
In cases, upon reset no lower blue bar is visible. This may occur if immediately afterwards the maximum is lost due to a wrong turn of one of the adjustment screws (see next step). A click on the reset button on the right moves the lower blue bar to the center.

7. Use the adjustment screws in the order left/right, up/down and back/forth. Use the first screw and check in which direction to turn in order to increase the length of the blue bars. Turn as long as the reading increases and then switch to the second screw and repeat the procedure. Then switch to the third one and do the same. Usually it is sufficient to go through this routine two or three times. Mind that the automatic resetting of the bars does NOT indicate a worsening of the adjustment (see step above)!



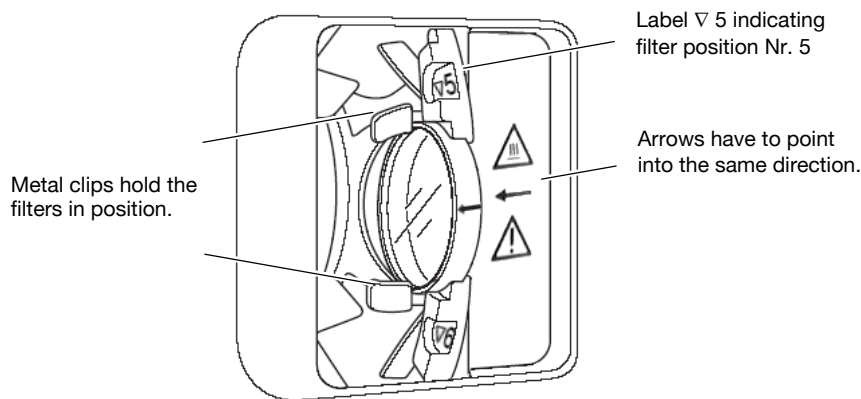
8. **Display warning message.** You can activate a display warning message to remind you that the burner is still turned on if you did not switch it off before exiting the scan^R Acquisition Software (see bottom of the OBS System Configuration screenshot at the beginning of this chapter).

4.3.3 Filter Exchange

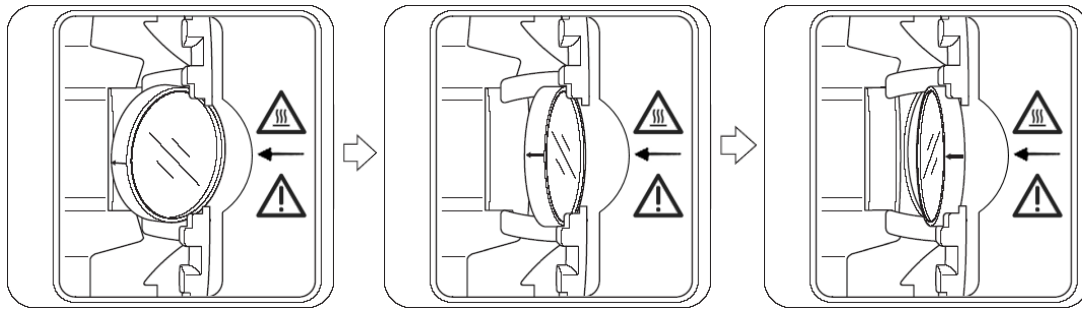


1. Start the **OBS System Configuration** software as described in the chapter above and go to the **Excitation Filter** page. Select the filter position that you want to exchange with the slider bar.
2. Open the flap at the right side of the MT20. The selected filter position is now accessible and allows the easy removal or insertion of a 25 mm filter. The filter wheel position is indicated by labels.

i The filters are held in place by metal clips. Insertion is easiest if the filter is moved into position tilted from the left side and then turned as shown in the series of drawings below. It is thus moved under the clips and sits tightly. Likewise, the easiest way to remove a filter is to turn it left and then pull it out. The left drawing below shows a filter moved halfway into position, still tilted to the left.



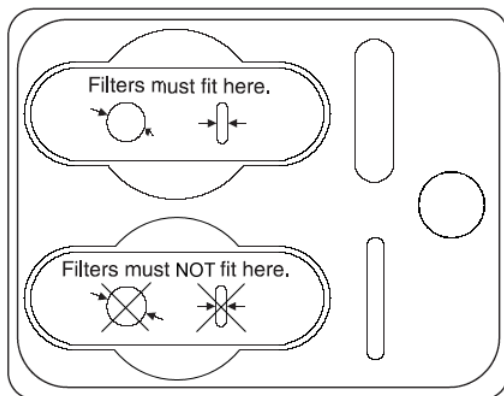
i Many filters are coated on one side and their performance is direction dependent. Make sure to insert the filters so that the little arrow on its frame points into the same direction as the arrow engraved on the right side of the filter wheel compartment.



3. Close the flap. The system will perform a mechanical initialization that takes about 18 seconds. If you listen closely you can hear the filter wheel and attenuator disk turn. After initialization the **STATUS** LED is turned on automatically to indicate that the system is ready.

i All filters provided by Olympus Soft Imaging Solutions have the standard size and fit into the filter wheel. In case you use other filters you should check their thickness and diameter using the templates on the inside of the filter flap.

Filters should not exceed the size given by the templates in the top row (**Filters must fit in here.**) nor should they be so small that they would fit into the templates in the lower row (**Filters must NOT fit in here.**). The slits on the side are for testing the thickness of the filters. The same rules apply here as for the diameter.



i Note that the filter wheel cannot be used via software for safety reasons while the flap is open. It can be moved by hand but a certain mechanical resistance has to be overcome.

Note that all changes to the Illumination System MT20 (besides filter and burner exchange) have to be done by Olympus Soft Imaging Solutions. Use optional equipment provided or approved by Olympus Soft Imaging Solutions only. Usage of other equipment may impair the performance or cause damage to the system. Safety of the system cannot be guaranteed in that case. Any damage to the system resulting from such action will void the warranty.

4.4 Centering the Illumination

1. Put a fluorescent sample onto the microscope stage. Most suitably the fluorescence covers the entire field-of-view.

2. Start the scan^R acquisition software and go to **Edit Scan ▶ Acquisition**.

3. Move a suitable filter cube in the microscope into position.

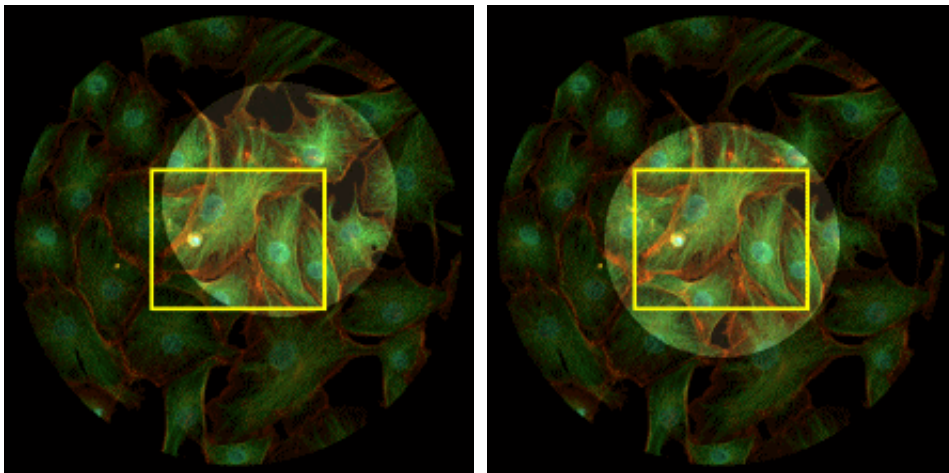
4. Select the corresponding excitation filter of the Illumination System MT20.

5. Make sure that the dispersion filter mounted in the filter slider (see 7 in photo in next chapter) of the illuminator is NOT in the light path.

6. Click the **Live settings** button.

7. Click the **Ocular view** button in the **Live view** window that opens.

8. Look through the eyepiece. You might find that the bright illumination circle is off center as shown in the left image below.



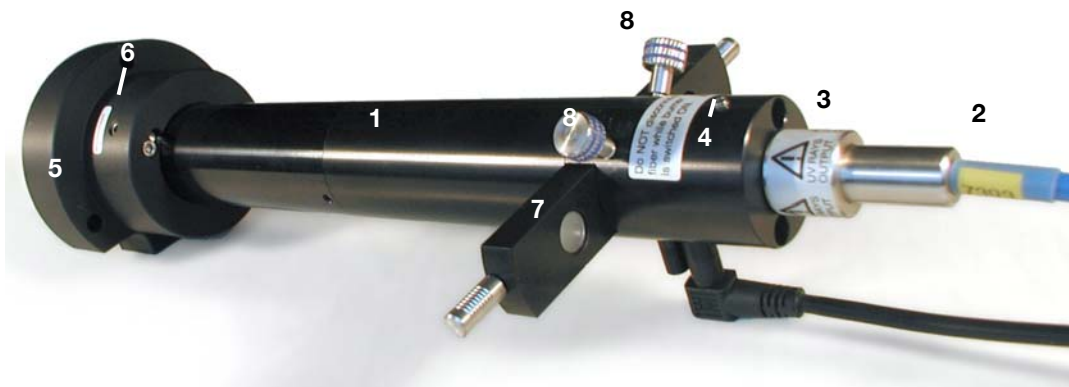
9. If you chose to observe the adjustment via the live view of scan^R (see step 6), you will get an image similar to the one inside the highlighted rectangle in the sketch above, which corresponds to the field-of-view of the 2/3" CCD chip of the camera.

10. Iteratively center the bright disk by turning the two adjustment screws at the epi-fluorescence illuminator (see 8 in the photo in the next chapter).

4.5 Optimizing the Illuminator Tube Position

For an optimally homogeneous illumination of the specimen, it is necessary for the light cone leaving the illuminator exit lens to match the position of the objective back aperture. Radial intensity gradients will result in case the distance between exit lens and back aperture is severely off; the images are brighter in the center than at the periphery.

Furthermore, in order to achieve maximum brightness, the exit plane of the light fiber has to be focused onto the specimen plane. This depends on the distance between the fiber exit and the illuminator optics, in other words, the depth of insertion of the light fiber into the illuminator. It is roughly optimized before delivery of the system by positioning the stopper ring **3** that sets the depth of penetration of the fiber port adapter. Usually this will be sufficient for a good illumination. However, if it is obviously defocused the light fiber position has to be re-adjusted by slightly turning the stopper ring. To be able to do that, the set screw **4** of the illuminator tube has to be slightly loosened by about half a turn.

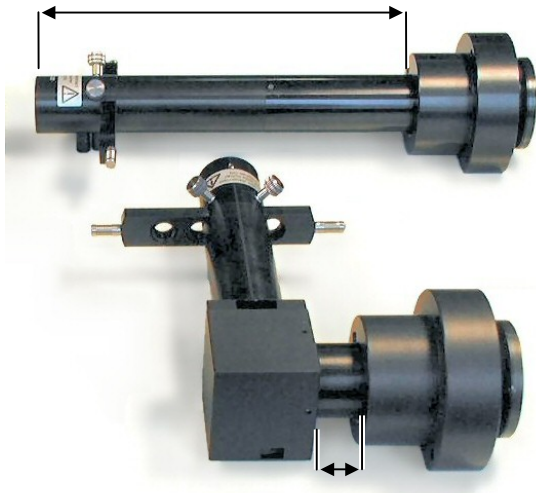


- | | |
|--|---|
| 1: illuminator tube containing the optical elements | 5: flange for IX2 microscope frame |
| 2: light guide with port adapter | 6: screw to fix the illuminator tube inside the flange |
| 3: stopper ring to adjust depth of penetration | 7: filter slider with dispersion filter |
| 4: set screw to fix the light guide | 8: adjustment screws for centering the illuminated field |

The optical path length, which is (partly) determined by the penetration depth of the illuminator tube **1** into the microscope frame, is likewise crucial. The penetration can easily be changed after slightly loosening the fixing screw **6** in the flange **7**. If the tube is either too far or not far enough inside the microscope, the overall brightness is reduced. However, the effects are hardly visible by eye, but they can be monitored by carefully analyzing the images.

The following table lists the optimal distances to be adjusted for the different types of illuminators. These distances are marked in the photo below. In the table you will find entries for the different groups of objectives.

It is inconvenient to change the illuminator position every time if objectives of all three groups are frequently used: here, the distance of group **B** objectives can be chosen as a compromise.



Standard and L-shape illuminators; the arrows mark the distances that have to be adjusted.

Optimal distance between flange and illuminator tube end [mm]		
Objectives	Standard	L-shape
A	231	41
B	221	31
C	211	21

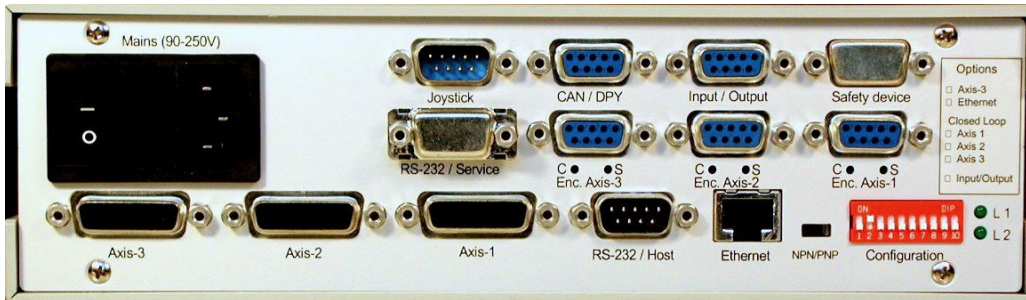
i The illumination will still be bright and homogeneous if, for example, a group **C** objective is used but the illuminator is installed with the group **A** distance. However, for experiments that are very critical with respect to the brightness of illumination, it might be useful to re-adjust the illuminator position.

Groups of objectives:

- A** LCPLFL 20x, LCPLFL 20x PH, PLAPO 1,25x, PLAPO 2x, UPLAPO 4x, UPLFL 4x, UPLFL 20x, UPLFL 4x P, UPLFL 20x P, UPLFL 4x PH, UPLFL 20x PH
- B** CPLFL 10x PH, LCPLFL 40x, LCPLFL 40x PH, LCPLFL 60x, LCPLFL 60x PH, PLAPO 40x, PLAPO 100x O3, PLFL 100x, SLCPLFL 40x, SLCPLFL 40x PH, UAPO 40x /340, UMPLFL 5x, UMPLFL 10x, UMPLFL 10x W, UMPLFL 20x, UMPLFL 40x, UMPLFL 50x, UMPLFL 100x, UPLAPO 10x, UPLAPO 10x W, UPLAPO 10x PH, **UPLAPO 20x**, UPLAPO 20x O, **UPLAPO 20x PH**, UPLAPO 40x, UPLAPO 60x, UPLFL 10x, UPLFL 10x PH, UPLFL 10x P, UPLFL 40x, UPLFL 40x P, UPLFL 40x PH
- C** all UIS2 objectives, APO100x OHR, LUMPLFL 40x W, LUMPLFL 40x W/IR, LUMPLFL 60x W, LUMPLFL 60x W/IR, PLAPO 60x O3, PLAPO 60x O3PH, PLAPO 60x O/TIRFM-SP, UAPO 20x /340, **UAPO 20x W/340**, UAPO 40x W/340, **UAPO 40x OI/340**, UMPLFL 20x W, UPLAPO 40x OI, UPLAPO 40x OI3PH, UPLAPO 60x W, UPLAPO 100x OI, UPLAPO 100x OI3PH, UPLFL 60x OI, UPLFL 60x OI3PH, UPLFL 100x O, UPLFL 100x OI, UPLFL 100x OP, UPLFL 100x O3PH, XLUMPLFL20XW

The objectives highlighted bold are the most sensitive with respect to an optimal adjustment. If they are frequently used, the distance should be set as corresponds to their group. If you cannot find a certain objective in this list, please contact your local Olympus specialist.

4.6 Connecting the Controller of the Motorized Stage



Back panel of the Corvus stage controller

In this description it is assumed that the motorized stage is mounted on the microscope frame as designed by the manufacturer.

1. Make sure that the main switch is set to OFF.

2. Set the dip switch 2 in the red **Configuration** field at the back panel of the controller to **ON** (up position). All other switches have to be in **OFF** position (down position). This sets the Baud rate (data transfer rate) to the correct value.



3. Connect the joy stick with the male 9-pin **Joystick** plug.

4. Connect the female 13-pin **Axis-2** plug with the y-axis motor (the one closer to the microscope frame) of the motorized stage.

5. Connect the female 13-pin **Axis-1** plug with the x-axis motor (the one closer to the edge of the stage) of the motorized stage.

6. Connect the male 9-pin **RS-232 / Host** plug with a 9-pin PC serial ports (COM1 or **PC-COM2**).

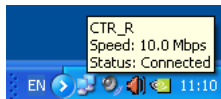
7. Make sure that the main switch is set to OFF and connect **Mains** with the power outlet.

i Do not use **CTR-COM2** serial port of the cell^R Real-time Controller. It is the PC that acts as host for the stage controller.

4.7 PC-to-Controller Network Connection

Communication between the MSWindows imaging PC and the scan^R Real-time Controller is realized through an ordinary, albeit internal, network connection via a network card.

The MSWindows status bar features an icon showing two little monitors. If the system works properly you will see the mouse-over message shown in the screenshot below:

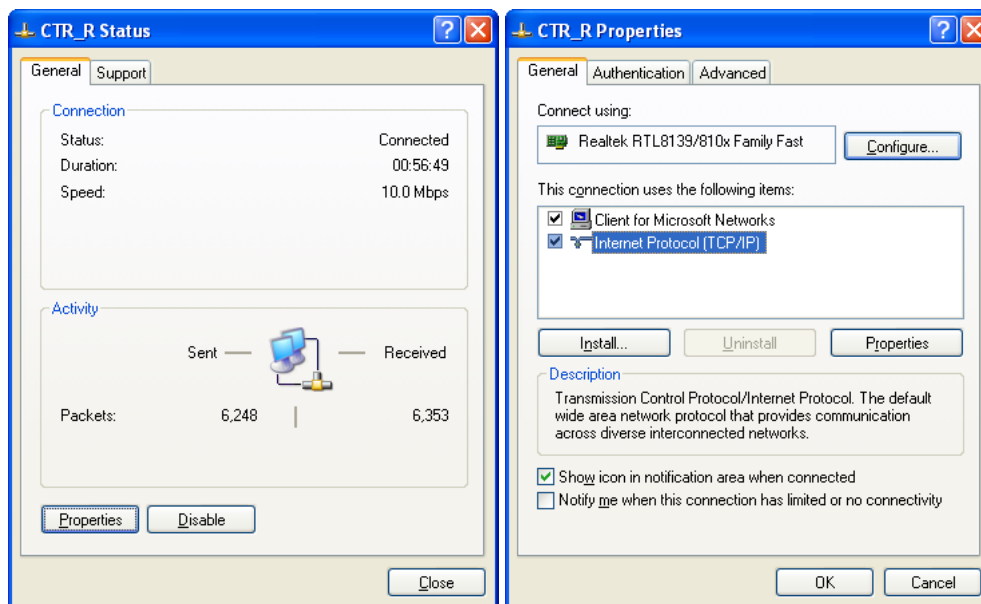


When double-clicking on the icon the **CTR Status** window will open. The **Activity** field counts the data **Packets** being **Sent** and **Received**.

It is important that the configuration of the PC-to-controller network connection remains unchanged. Otherwise the system may become disabled.

8. To control the settings, click on the **Properties** button in the **CTR Status** window to open the **CTR Properties** window.

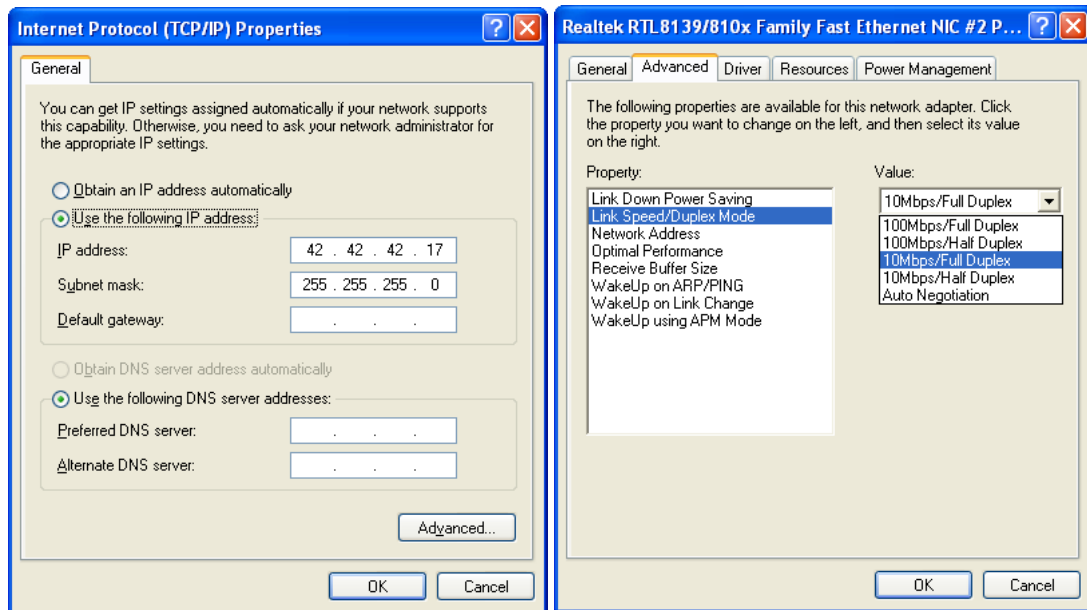
9. Select the *Internet Protocol (TCP/IP)* connection and click on the **Properties** button to open the **Internet Protocol (TCP/IP) Properties** window.



10. The **Use the following IP address** has to be activated.

11. The **IP Address** must be *42 42 42 17*.

12. The **Subnet Mask** must be *255 255 255 0*.



13. Return to the **CTR Properties** window with **OK**.

14. Click on the **Configure** button and go to the **Advanced** tab of the **Realtek** window that opens.

15. Select the **Property Link Speed/Duplex Mode** and then the **Value 10Mbps/Full Duplex** from the shortlist. Confirm with **OK**.

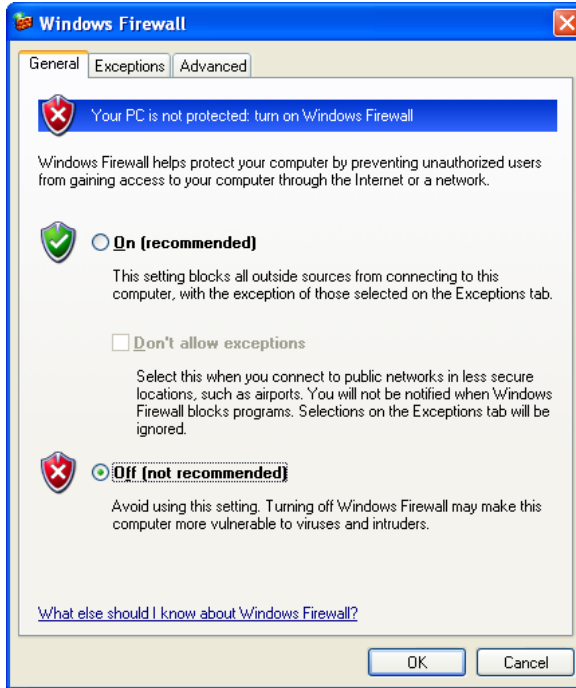
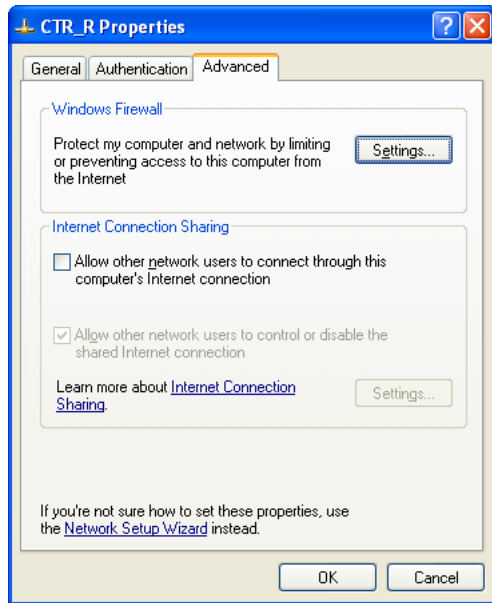
Additionally, it is important the PC-to-controller network connection is NOT protected by a firewall.

16. To control this, go to the **Advanced** tab of the **CTR Properties** window and click on the **Settings** button.

17. Make sure that **Off (not recommended)** is activated in the **Windows Firewall** window.



The PC-to-controller network connection is not a computer safety risk and there is no reason for a firewall protection. This internal network connection is used only for the system commands traffic and not a gateway to the internet or intranet.



OLYMPUS[®]

OLYMPUS LIFE SCIENCE EUROPA GMBH
Postfach 10 49 08, 20034, Hamburg, Germany

