OLYMPUS®

cell tirf

USER INSTRUCTIONS

MITICO(-2L)
LAS-BOB
LAS-MC

MOTORIZED INTEGRATED TIRF ILLUMINATION COMBINER
LASER SAFETY BREAK-OUT BOX
LASER MANUAL CONTROL

This instruction manual describes the Olympus cell tirf MITICO illumination combiner with accessories. To ensure safety, obtain optimum performance and familiarize yourself fully with the use of this product, we recommend that you study this manual thoroughly before operation. Together with this manual, please also read the imaging system manuals, the laser manual and the instruction manual of the microscope in order to understand overall operation methods. Retain this manual in an easily accessible place near a system for future reference.

OLYMPUS SOFT IMAGING SOLUTIONS GMBH
Johann-Krane-Weg 39
D-48149 Münster
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

Manual version: September 2010

Contents

1 Introduction 1
1.1 Abstract 2
1.2 General 2
1.3 Components 3

2 Safety Symbols 5

3 Safety Precautions and Handling Instructions 9
3.1 Laser Safety Precautions 10
3.2 General Safety Precautions 11

4 cell^tirf MITICO and Peripherals Overview 13
4.1 Setup Scheme 14
4.2 The Laser Safety Features 15
4.3 The MITICO Features – an Overview 17
4.4 The LAS-BOB Laser Safety Break-out Box 18
4.5 The Laser Shutter Manual Control 18
4.6 The AD-USB-ODB Bus Adaptor Box 19
4.7 The Remote Interlock Connector of the Olympus Soft Imaging Solutions ODB Laser Systems 20
4.8 The Light Path Selector Slider 20
4.9 The Iris Diaphragm Field Stops 21
4.10 Laser Clean-up Filter Sliders 22
4.11 Focusing the Laser Lines 22
4.12 The FRAP Lens of Laser Line 1 23
4.13 Laser Adjustment 23
4.14 Filters, Mirrors, Objectives 24

5 Modes of Operation 25
5.1 The User Mode 26
5.2 The Maintenance Mode 27
5.2.1 Exchanging Microscope or Camera Hardware 28
5.2.2 Adjusting the Laser Beam Position 28
5.2.3 Access to the Specimen without Deactivating the TIRFM System 28
5.2.4 Observing the Specimen under TIRF Illumination Through the Ocular 29

6 Using the cell^tirf MITICO Illumination Combiner 31
6.1 The cell^TIRF Control Software 32
6.2 cell^tirf MITICO Control via the cell^R Software 38
6.2.1 System Configuration 38
6.2.2 Operating cell^tirf MITICO 39
6.2.3 TIRFM in the Experiment Manager 41

7 Specifications and Technical Data 45

1 Introduction

Thank you very much for purchasing Olympus Soft Imaging Solutions' state of art motorized cellTIRF illuminator system, and for your confidence in our products and service. It is Olympus Soft Imaging Solutions' main objective to provide you with solutions able to meet your experimental demands and thus pave the way to your scientific success.

The cellTIRF illuminator system is designed for general laboratory use only. It allows coupling up to four lasers and a white-light source to an inverted Olympus IX2 microscope and to adjust the position of each laser beam individually relative to the optical axis to obtain total internal reflection illumination.

1.1 Abstract 2
1.2 General 2
1.3 Components 3

Motorized multi-port cell tirf illumination combiner MITICO with fiber port for widefield illumination

1.1 Abstract

In the following sections you will find a detailed description of the components of the cell tirf illuminator system, handling and alignment instructions and how to maintain the performance of your TIRF microscope. Keep in mind that all components are designed to work together with an Imaging Station like the Olympus cell^M or cell^R as an integrated system. We strongly discourage taking away or replacing single components without prior consultation of Olympus Soft Imaging Solutions, most probably it will impair the performance of the system dramatically. The guarantee will not cover any damages to the system due to such mishandling. All necessary tools for handling and alignment (except the laser safety goggles) are part of the product.

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

1.2 General

The MITICO motorized integrated TIRF illumination combiner is compatible with the Olympus microscopes IX51, IX71 and IX81 as well as a range of laser systems within visible spectral range between 400nm and 700nm provided that they meet the following requirements:

- Every laser unit must have an input remote-control safety interlock that must be connected to the central laser emergency stop and which interrupts the laser radiation in case of danger (EN 60825-

1:2003, Sect. 4.4 and 10.2). Furthermore, every laser unit must have a key-activated main power switch in the sense of EN 60825-1:2003, Sect. 4.5).

- Provided that the laser units can be operated independently they must have their own emission warning display (audible or visible) (EN 60825-1:2003, Sect. 4.6).
- The combined power of all employed and/or connected laser powers must not exceed 500mW.

This instructions manual describes the hardware components of the cell^TIRF illuminator system, their installation and alignment. For the use of the connected lasers refer to the respective manuals.

It is important to familiarize yourself with the entire system to ensure safety and optimum performance. We recommend studying this manual as well as the software and hardware manuals of the imaging system thoroughly before operating the cell^TIRF illuminator system. Keep this manual close to the imaging station for future reference.

The cell^TIRF illuminator system meets the CE requirements:

- EN61326 Class A
- EN60825

1.3 Components

The TIRF illuminator system consists of the following hardware components:

a MITICO motorized integrated TIRF illumination combiner:
- Four fiber ports, each with adjustable line focus
- A port for either a TIR-LHA adaptor to couple a standard fluorescence lamp housing or TIRMT-CON to couple a MT10 / MT20 illumination system.
- A slider to switch between 100% widefield illumination and 50% widefield plus 100% TIRF
- Motorized drives to move each laser line
- A field stop for each laser line
- Removable slider for a clean-up filter for each laser line
- FRAP focus lens for the first line that can be moved in and out of the optical path
- Laser safety shutter with key
- Illumination combiner support

b Laser shutter hand switch LAS-MC with key with emergency interrupter and key-controlled switch to maintenance mode

c Laser safety break-out box LAS-BOB

d Laser safety stage cover with translucent laser safety glass that allows following the beam position

e Laser safety ocular shutter

f Laser beam alignment tool

g Manuals

The following items are additionally needed to convert a fluorescence microscope into a TIRF microscope:

h Up to four laser systems with a maximum total of 500 mW light output

i – Either a TIR-LHA adaptor to couple a standard fluorescence lamp housing
– or a TIRMT-CON to couple a MT10 / MT20 illumination system

j A widefield illumination system
– Either a standard fluorescence lamp housing, e.g., U-LH100HGAPO
– or a MT10 / MT20 illumination system

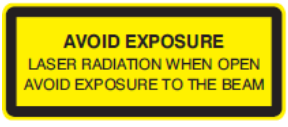
2 Safety Symbols

The deactivation of the safety systems or the dismantling of different components by the operator is required in certain operational modes and in case of illumination alignment. As a result the Olympus motorized cell^TIRF illuminator system is to be classified in accordance with EN 60825-1:2003 in **laser safety class 3B** and in accordance to EN 954-1:1997 in **hazard category 2**. Class 3B lasers are medium power lasers with an output power of up to 500 mW within 400 – 700 nm wavelength. Laser safety regulations require extensive labeling of all possible apertures where laser radiation might potentially exit including such apertures that are totally covered except upon partially or totally disassembling the system.

The following symbols are placed on the Olympus cellTIRF illuminator system. Study the meaning of the symbols and always use the equipment in the safest possible manner.

General symbols	
Symbol	**Explanation**
	Indicates the use of a laser beam. Take special care in handling that part. (See also following chapter)
	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.
 **EU Only**	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

Each laser source must be provided with a danger symbol, a sign with the classification text and a sign on the beam exit opening. Please ensure that your microscope is labeled as shown in the table below. For all parts labeled with the following labels special laser precautions must be considered (see Chapter 4.1, *Laser Safety Precautions*)!

Laser safety symbols		
Symbol	**Explanation**	**Location**
	Indicates the use of a laser beam. Take special care in handling this part.	Front of the MITICO illumination combiner; see photos **1**
	Laser radiation can cause irreversible damage to your eyes. Wear adequate protection before opening this aperture.	Front of the MITICO illumination combiner. May be hidden if the MITICO is mounted on the microscope; see photo **1**. Microscope frame, right side, left side near C-mount; see photos **2** and **3**.
	These apertures should be closed while operating the system. Parts connected should be dismounted by trained personal only and when the laser is turned off.	Laser safety ocular shutter; see photo **4**.
	When system is switched to maintenance mode laser radiation can be emitted from this aperture. Be sure to wear adequate eye protection.	Laser safety stage cover; see photo **5**.

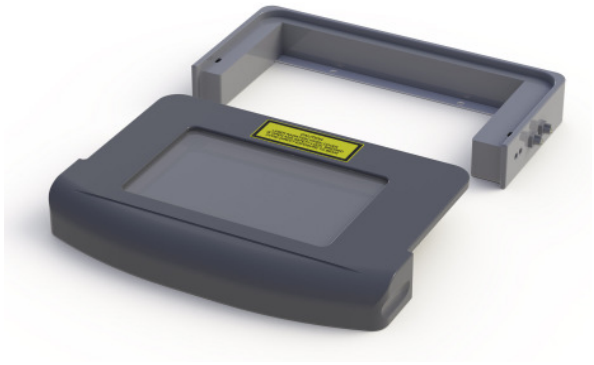
Labels for the microscope components are included in the shipping list of the cell^tirf illuminator system. Please paste them on the microscope upon system installation. If any label is missing please ask your Olympus representative for additional labels.

1: cell^tirf MITICO motorized integrated TIRF illumination combiner

2: microscope frame, right side

3: fluorescence turret and C-mount

4: laser safety ocular shutter



5: laser safety stage cover

3 Safety Precautions and Handling Instructions

The Olympus cellTIRF illuminator system must be used with laser products belonging to the Laser Safety Class 3B. Class 3B lasers are medium power lasers with an output power of up to 500 mW within 400 – 700 nm wavelength. The total of the output power of the connected lasers may not exceed 500 mW. Viewing these lasers under direct beam and specular reflection conditions is hazardous. The diffuse reflection is usually not a hazard except for higher power Class 3B and Class 4 lasers. Normally the class 3B laser is not a fire hazard. A class 3B laser product may only be used under the control of a laser safety officer. Before using this product be sure to be familiar with the demanded safety measures according to EN 60825-1 and the national regulations

3.1 Laser Safety Precautions 10
3.2 General Safety Precautions 11

3.1 Laser Safety Precautions

> Caution: Control or adjustments using procedures other than those prescribed in this manual will lead to hazardous laser beam exposure.

To avoid hazardous situations strictly follow the laser safety instructions below.

1. Before operating an Olympus cellTIRF illuminator system check if all devices are attached correctly and all apertures are closed.

2. The usage of a Class 3B laser is only permitted under the control of a **laser safety officer**.

3. A Class 3B laser **can cause irreversible damage to your eyes**. Thus do not stare into the laser beam or illuminated fiber. Make sure to wear adequate eye protection during operation of the laser in maintenance mode.

4. Always operate the cellTIRF illuminator system in **user** mode unless you underwent a detailed training in operating the system in **maintenance** mode. Inform your laser safety officer if you intend to operate the system in **maintenance** mode. The **user** mode is the safest manner to operate the system. Opening the stage cover or the binocular slider will interrupt the laser beam in this mode.

5. Only trained Olympus service personnel is allowed to perform the installation and alignment procedures described in the Service Manual.

6. A person in charge with the laser alignment or performing scientific experiments while operating the laser in **maintenance** mode must be aware of the safety precautions adequate to the TIRF microscope setup. He/she is responsible for the safety of any person present in the room while operating the lasers in **maintenance** mode.

7. The focused laser beam or any UV light emitted from the microscope nosepiece **may injure the skin** through the objective exit lens and through a free nosepiece position. Thus, avoid exposing the hands to the laser beam when working on the microscope.

8. Do not bend, step on or pull excessively on the laser fibers. This could damage a fiber and cause hazardous beam leakage. In case of a damaged fiber, switch off the laser immediately and contact Olympus.

9. Be sure all apertures of your microscope system stay closed or are connected while operating the laser. A change in camera or any microscope part should be performed only when the laser is turned off.

10. Never cover the air outlet of the laser to prevent overheating.

Direct exposure to the laser light is possible at the following positions:

- The laser system's housing at the fiber port if the fiber is removed
- The exit of the laser fiber
- The fiber coupling of the widefield illumination system to the cellTirf illuminator system
- The beam path of the microscope
- Any microscope port, which includes the entrance ports because the laser light can be reflected within the microscope
- The light output port of the widefield illumination system
- The objective tip or – if no objective is mounted – a free nose piece position

3.2 General Safety Precautions

a Always use power cords and power supplies provided or approved by Olympus Soft Imaging Solutions or the manufacturer of your laser system.

b Provide unimpaired access to the emergency shutter switch at the LAS-MC manual control and to the main power switches.

c Only those lasers may be connected that comply the laser safety regulations as described in Chapter 1.2.

d Always make sure that the grounding terminal of the laser system and the wall outlet are connected properly. If the system is not grounded, Olympus Soft Imaging Solutions cannot warrant the electrical safety and proper performance of the product.

e Please refer to the user manuals of the respective laser manufacturer for adequate safety precautions and proper usage.

f If the equipment is not used as specified in this manual, the safety and performance may be impaired. In addition the equipment may be damaged and warranty may be lost. Use the equipment only as outlined in this instruction manual.

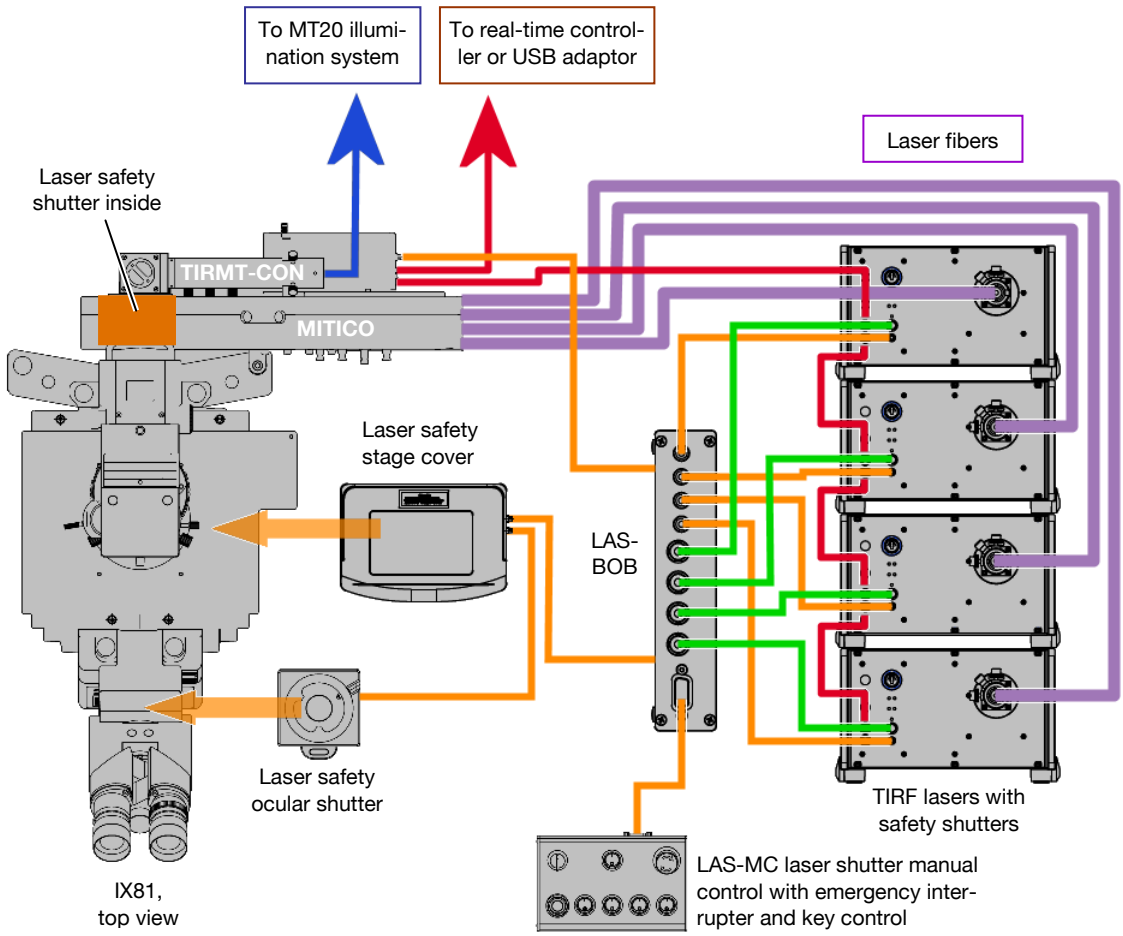
Olympus Soft Imaging Solutions GmbH accepts no liability for any health damages caused by improper use of the lasers and laser related setup!

4 cellTIRF MITICO and Peripherals Overview

The following chapter describes all components and interfaces of the Olympus cellTIRF illuminator system including all laser safety devices.

4.1 Setup Scheme 14
4.2 The Laser Safety Features 15
4.3 The MITICO Features – an Overview 17
4.4 The LAS-BOB Laser Safety Break-out Box 18
4.5 The Laser Shutter Manual Control 18
4.6 The AD-USB-ODB Bus Adaptor Box 19
4.7 The Remote Interlock Connector of the Olympus Soft Imaging Solutions ODB Laser Systems 20
4.8 The Light Path Selector Slider 20
4.9 The Iris Diaphragm Field Stops 21
4.10 Laser Clean-up Filter Sliders 22
4.11 Focusing the Laser Lines 22
4.12 The FRAP Lens of Laser Line 1 23
4.13 Laser Adjustment 23
4.14 Filters, Mirrors, Objectives 24

4.1 Setup Scheme



Scheme of an IX81 microscope with a cellSens MITICO illumination combiner with widefield illuminator TIRMT-CON, four lasers and the laser safety equipment .

- Connections of the laser safety devices
- ODB communication connections
- TTL trigger connections to switch the functional laser shutters via LAS-MC
- MT20 light fiber
- Laser fibers

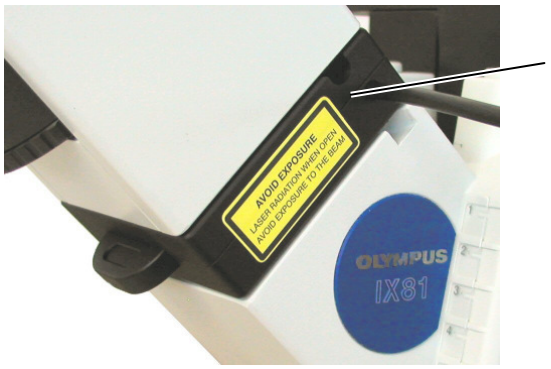
4.2 The Laser Safety Features

The laser safety concept of the cell^TOP illuminator system consists of the following modules:

- A double-redundant laser safety shutter in front of the exit optics of the cell^TOP illumination combiner
- A laser safety ocular shutter
- A laser safety stage cover
- An emergency interrupter located on the LAS-MC manual control
- The double-redundant laser safety shutter of Olympus laser systems
- The LAS-BOB laser safety break-out box that connects all devices electronically

Be sure that all listed components are correctly mounted:

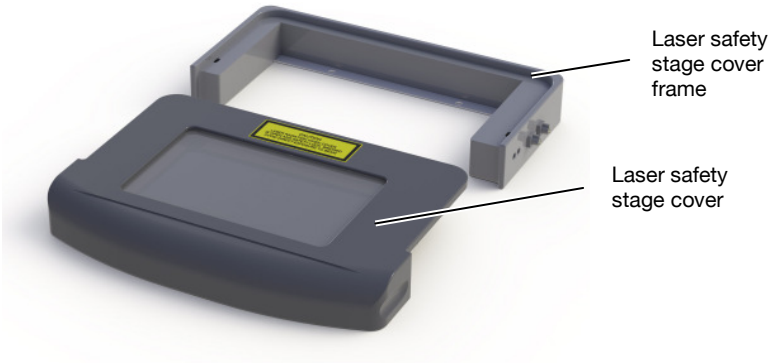
- The laser safety ocular shutter must be placed in the ocular light path as shown below.



Laser safety ocular shutter

- The U-shaped frame of the laser safety stage cover must be fixed on the stage and the stage cover in place.

The laser safety stage cover must be secured to the microscope stage. If the microscope stage is not equipped with the necessary pre-drilled holes please make sure that this modification is performed before using the system. Appendix –Laser Safety Stage Cover Dimensions in the cell^TOP **Service** Manual provides drawings with all dimension data necessary for such a modification.



- The 5-pin ocular shutter cable must be connected to the stage cover and this additionally to the 5-pin **STAGE COVER** plug of LAS-BOB laser safety breakout box.
- One of the two **SAFETY SHUTTER** plugs of the LAS-BOB laser safety break-out box must be connected with the laser safety shutter plug on the electronics housing on the back side of the MITICO illumination combiner using the 4-pin cable.

Check the safety shutter function in user mode in a completely installed TIRFM system.

1. Turn the key in the LAS-MC manual control to **USER MODE**.

2. Set the **REMOTE** switch of the LAS-MC manual control to **0**.

3. Open the shutter of one of the connected lasers with the corresponding **LASER** switch on the LAS-MC manual control. You should observe laser light through the translucent stage cover insert.

4. Open the ocular shutter slider. The indicator diode on the laser safety shutter must become switched off immediately indicating the closure of the shutter. No laser light should be visible anymore. Close the slider again.

5. Carefully lift the lid of the stage cover. The indicator diode on the laser safety shutter must become switched off immediately indicating the closure of the shutter. No laser light should be visible anymore. Mount the cover again.

The LAS-MC manual control features an emergency interrupter, the red button. If the system is properly setup and you press the red button,

- The safety shutter of any Olympus Soft Imaging Solutions laser system connected must close
- Any other laser connected to the LAS-BOB break-out box must be switched off entirely
- The safety shutter of the MITICO illumination combiner must close.

In order to reactivate the system you have to turn the emergency interrupter to release it from the locked position.

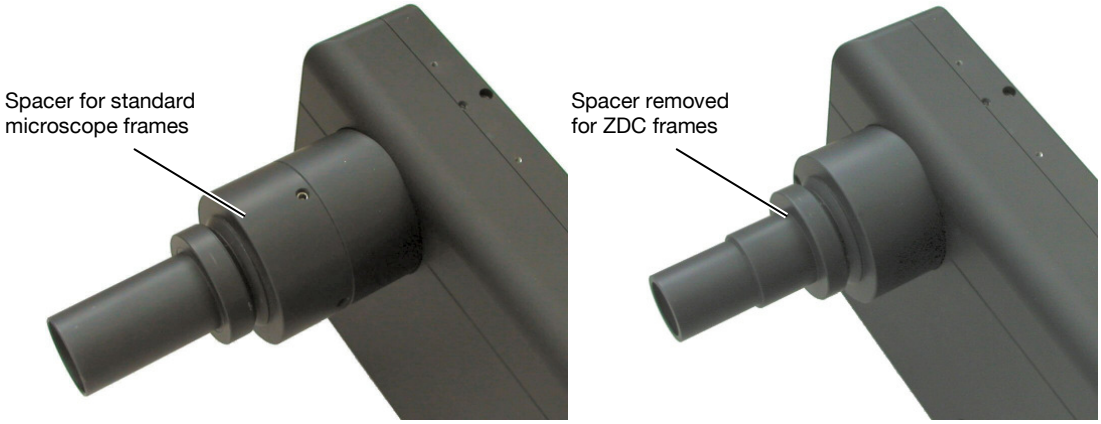
> Applying the laser safety emergency interrupter is not a standard test and should only be tested if changes to the laser configuration and connections have been made. It is only for emergency use and will cut the power supply of any laser except the Olympus Soft Imaging Solutions laser systems.

If you have doubts or questions concerning your laser safety devices, contact your laser safety officer.

4.3 The MITICO Features – an Overview

The MITICO motorized integrated TIRF illumination combiner features

- Four FCP8/FCP laser fiber ports with four-axis alignment
- Two Lemo plugs to connect it via ODB communication cables with
  a) – either the real-time controller inside the PC of a cell^M / cell^R imaging system
  – or the AD-USB-ODB bus adaptor box and
  b) other OSIS ODB devices (in daisy chain)
- A 5-pin plug to connect with the **SAFETY SHUTTER** plug of the LAS-BOB laser safety break-out box
- Two red safety shutter status diodes
- A green ODB communication status diode
- A green power status diode
- An iris diaphragm field stop for each laser line
- A laser clean-up filter slider for each laser line
- A laser beam focus slider for each laser line
- A switch to insert a FRAP focusing lens into the beam path of line **1**
- A light path selector slider to switch from 100%TIRF with 50% widefield illumination to 100% widefield illumination
- A widefield fluorescence illumination port to mount
  a) either a TIRMT-CON widefield fiber illumination coupling if a fiber-coupled fluorescence illumination systems MT10 or MT20 is to be used or
  b) a TIR-LHA adaptor if a standard fluorescence lamp housing is to be used
- An epi-fluorescence illuminator tube to connect it with the microscope frame. This contains a spacer unit with tube, which has to be removed for ZDC-equipped microscopes.



4.4 The LAS-BOB Laser Safety Break-out Box

It features on the front panel

- Four **INTERLOCK 4 – 3 – 2 – 1** cinch plugs to connect it with the **INTERLOCK** connectors of Olympus Soft Imaging Solutions laser systems (Other lasers may need adaptors.)
- Four **SHUTTER 4 – 3 – 2 – 1** BNC TTL-OUT plugs to connect with TTL-IN ports of Olympus Soft Imaging Solutions laser systems or laser shutters like the LAS-SHU-PSFIB.
- A **LAS-MC** D-sub 15-pin female plug to connect with the laser shutter manual control

and on the back panel

- Two **SAFETY SHUTTER** 4-pin female plugs one of which is to be connected with the corresponding plug on the MITICO illuminator
- Four **DIGITAL I/O 1 – 2 – 3 – 4** BNC TTL-IN plugs to connect with shutter control TTL-OUT ports of a control unit, for example the cell^M / cell^R real-time controller in case that no laser systems by Olympus Soft Imaging Solutions are used
- A **STAGE COVER** 4-pin female plug to connect with the laser safety stage cover
- A **24 V** ⎓ ⊖⊂⊕ DC power supply plug to connect with 24 V DC power supply.

4.5 The Laser Shutter Manual Control

The laser shutter manual control features

- A D-sub 15-pin female plug to connect with the **LAS-MC** plug of the LAS-BOB break-out box
- Four **LASER 1**, **2**, **3**, **4** switches to open and close each high-speed laser shutter individually
- A **REMOTE** switch to set the shutters to remote control via external TTL triggers or ODB device bus communication (typically controlled by a software like cell^M / cell^R)
- A mode lock with key to allow authorized and trained personnel to switch from standard **USER MODE** to **MAINTENANCE** (see Chapter 5, *Modes of Operation*).
- A red emergency interrupter that close all safety shutters when pressed

– A **BINOCULAR** button to bridge the laser safety ocular shutter in **maintenance** mode so that the specimen can be observed via ocular with laser illumination. This is not a toggle button; it needs to be kept pressed in order to keep the laser safety shutter open.

> ⚠ Be extremely cautious when bridging the laser safety ocular shutter by pressing the red button! Be sure that a filter cube that quantitatively blocks reflected laser light is inserted into the light path. Otherwise hazardous and intense laser light might exit the ocular. Set the laser to a low intensity with the attenuator before first looking through the ocular.

4.6 The AD-USB-ODB Bus Adaptor Box

It is needed for non-cell^M / cell^R systems only and features on the front panel:

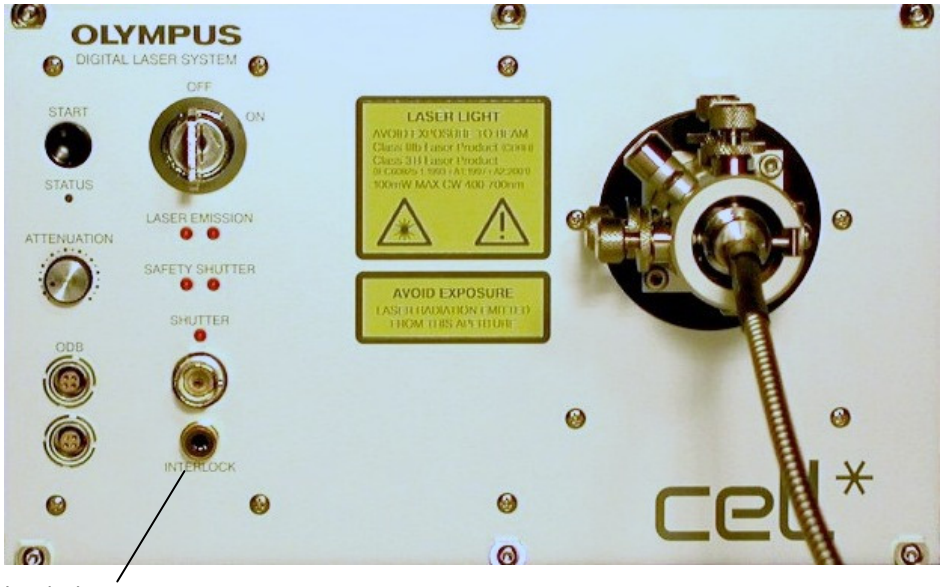
– A power On/Off switch.
– A green **Power** On diode
– An orange **ODB** communication status diode

and on the back panel:

– An **ODB** plug to connect it via ODB communication cables with the MITICO illuminator
– A **USB** Type-B plug to connect it with the imaging PC via a USB Type-A-to-Type-B cable
– A **24 V** ⎓ ⊖-◉-⊕ DC power supply plug to connect with 24 V DC power supply.

4.7 The Remote Interlock Connector of the Olympus Soft Imaging Solutions ODB Laser Systems

An **INTERLOCK** connector can be found at the front panel of all Olympus Soft Imaging Solutions laser systems as Class 3B laser products. This remote interlock connector must be connected to the emergency interrupter of the system via the corresponding **INTERLOCK 4 – 3 – 2 – 1** cinch plug of the LASBOB break-out box.



Interlock connector

Caution. Class 3B laser products not in use must be protected against unauthorized use by removal of the key from the key control.

It is recommended that the interlock connectors of non-Olympus Soft Imaging Solutions laser systems be connected in the same way.

4.8 The Light Path Selector Slider

The Olympus cellTIRF MITICO illumination combiner features the possibility to combine widefield imaging via a white-light illumination system with laser illumination for TIRF applications.

A polarizing beam splitter cube has to be moved into the light path to enable both types of illumination in one experiment. This is done with the light path selector slider located on top of the MITICO illumination combiner.

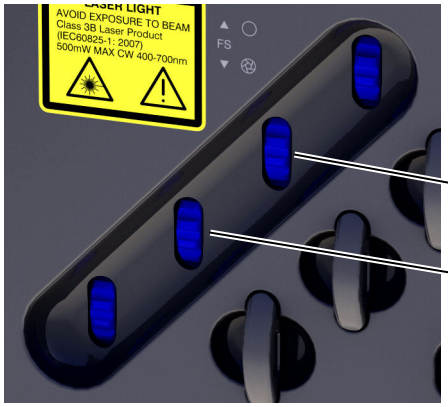
The slider has to be moved all the way to the right to operate either with the TIRFM laser only or to combine TIRFM illumination with widefield illumination. The polarizing beam splitter cube blocks 50% of the widefield illumination intensity but retains intensity of the polarized laser beam quantitatively.

The slider should be moved all the way to the left to remove the polarizing beam splitter cube from the light path and thus to get the maximum possible intensity from the white-light illumination system in experiments that require widefield illumination exclusively. A glass element is moved into the light path to maintain the optical path length.

Light path selector slider. **WF** ◄ (left) : 100% widefield; ► **TIRF/WF** (right): 100% TIRF, 50% widefield

4.9 The Iris Diaphragm Field Stops

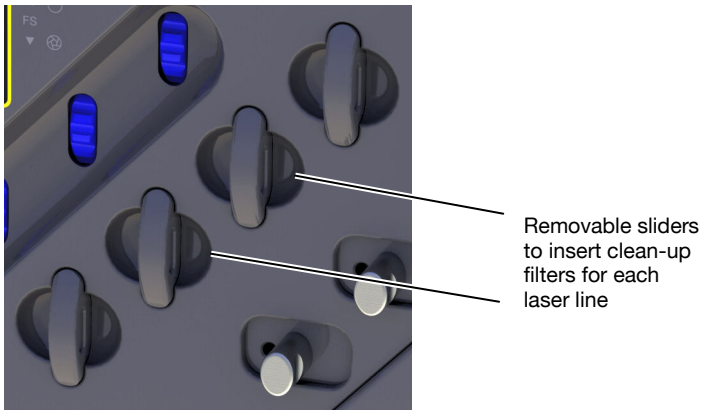
The cellTIRF MITICO illumination combiner contains infinitely variable iris diaphragm field stops for each laser line to adjust the area of illumination.



Thread screws to adjust the field stop diaphragms of each laser line.
▲: open
▼: close

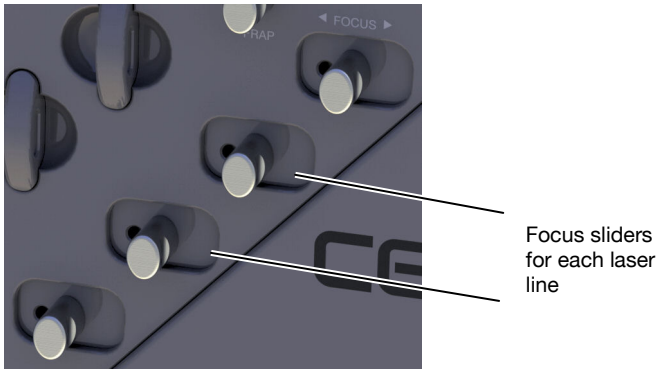
4.10 Laser Clean-up Filter Sliders

Some lasers may require clean-up filters to suppress background light. Standard 25 mm clean-up filters can be inserted into the respective slider of each laser line in the MITICO illumination combiner. The sliders can be removed and inserted easily.



4.11 Focusing the Laser Lines

In order to obtain the best possible TIRF performance it is necessary to focus each laser beam into the back focal plane of the objective. This ensures that a narrow, collimated beam exits the objective. This is the prerequisite for a pronounced transition from widefield to TIRF illumination and a good Z resolution.



In order to check the focusing, do the following:

1. Move the TIRF objective and a suitable mirror cube into position without any sample mounted and the laser safety stage cover in place.

2. Set the laser lines to widefield position via the TIRF control window; see Chapter 6.

3. Open the laser shutter and observe the beam as it transmits the translucent insert in the laser safety stage cover.

4. Unlock the fixing screw to the left of the focus slider of the cellTIRF MITICO illumination combiner.

5. Move the slider back and forth until the position is found where the laser spot is the smallest.

6. Close the laser shutter and tighten the fixing screw.

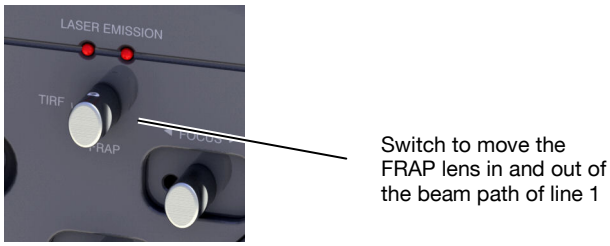
4.12 The FRAP Lens of Laser Line 1

In a perfectly aligned system the laser beams are focused into the back focal plane of the objective. Thus a parallel beam exits the objective with a width that corresponds to the field of view.

Line 1 of the cellTIRF MITICO illumination combiner features an additional lens that can be moved into the beam path. It causes the focus of the laser beam to shift from the back focal plane into the object plane (or focal plane). Here the size of the beam is diffraction limited if perfectly focused and appears as a tiny bright spot in the images. Move the focus slider of line 1 to enlarge the size of the illuminated spot; see Chapter 4.11, *Focusing the Laser Lines*. This feature allows to use line 1 for simple non-scanning FRAP experiments and other bleaching or photo-activation applications.

TIRF mode: turn the switch clockwise to move the lens out of the beam path.

FRAP mode: turn the switch counterclockwise to move the lens into of the beam path.



4.13 Laser Adjustment

If illumination in total internal reflection mode cannot be achieved, the reason might be a problem with the laser alignment. Contact your laser safety officer or the Olympus service to check the laser adjustment in maintenance mode as described in the cellTIRF service manual. Only trained Olympus service personnel are allowed to perform the alignment.

Be sure to wear the adequate eye protection for the laser wavelength and laser power in use. Once the cellTIRF MITICO illumination combiner is aligned, be sure to switch back to **User Mode** to activate the safety devices. According to the regulations trained personnel only are allowed to maintain a Class 3B laser system may conduct use of the system under Maintenance mode.

4.14 Filters, Mirrors, Objectives

Using the adequate optical elements with your TIRFM setup is essential for proper functioning. Furthermore, it can be dangerous to have filters or mirrors in use that are not adequate for your setup. The filter cubes used for TIRF experiments are in general not equipped with an excitation filter because the laser light is monochromatic, thus rendering excitation filters unnecessary.

Any excitation filter in the light path of a TIRFM setup, i.e., in the mirror unit turret of the microscope, may lead to interference effects and improper results.

Important for the safety in maintenance mode is the use of a proper emission filter and a dichroic mirror that blocks the laser (excitation) light returning from the specimen upon total reflection at the cover slip-water interface. Because of the incident light being totally, i.e., quantitatively, reflected at the cover slip-water interface when the system is in TIRF mode, the demands on the optical performance of dichroic mirror and emission filter are much higher than for standard widefield illumination. Thus, typical filter combinations used for standard fluorescence applications often cannot be used for TIRFM even if the excitation wavelengths coincide.

Several Olympus objectives are especially designed for TIRFM. The PLAPON 60XO TIRFM, the PLAPO 100XO TIRFM objective and the UAPO 150XO TIRFM (all NA = 1.45) as well as the APON 60XO TIRFM (NA = 1.49) can be used with ordinary immersion oil. The APO 100X OHR objective with its extremely high NA = 1.65 offers a wider range of TIRF incident angles and thus allows a certain tuning of the depth of the evanescent field. However, special and expensive high-refraction cover glasses (n = 1.78) have to be used as well as a matching immersion oil (di-iodo methane, which is harmful) to match the extreme objective NA. The standard oil immersion PLAPON 60XO objective has an NA of 1.42, which is slightly above the minimum required for TIRF, and thus only of limited use for TIRFM applications.

Caution: Cover the unused positions of the revolver nosepiece with adequate lids to prevent unwanted laser output.

5 Modes of Operation

There are two modes of operation, the **user mode** and the **maintenance mode**. In user mode no laser radiation can exit the system. The maintenance mode is for setting-up and adjusting the system. Only trained personnel are allowed to operate the system in maintenance mode.

5.1 The User Mode 26
5.2 The Maintenance Mode 27
5.2.1 Exchanging Microscope or Camera Hardware 28
5.2.2 Adjusting the Laser Beam Position 28
5.2.3 Access to the Specimen without Deactivating the TIRFM System 28
5.2.4 Observing the Specimen under TIRF Illumination Through the Ocular 29

5.1 The User Mode

All hardware has to be connected properly to operate the system in **User mode**. The user mode ensures that the user can operate the system without being exposed to the laser beam. It is possible to have full control of the various devices via software or manually. In **User mode** the system can be used like a laser of Laser Safety Class 1.

Actions in user mode		
Permitted Action	**Remarks**	**Actions not permitted**
Change or manipulation of specimen with laser safety stage cover lifted	Safety shutter closed automatically	
Execution of Experiment Plans in cell^M / cell^R or similar processes in other imaging software		
Live observation via camera, image acquisition in TIRF conditions		Observation via binocular
Change of camera settings and switch of microscope modules as long as no hardware is being dismounted	Best with safety shutter closed	Exchange of microscope or camera hardware
Change of filters or burner in the illumination system MT10 / MT20 or other filter switching devices		Detaching the optical fiber
Change of burner in a fluorescence lamp housing (if used)		Detaching the lamp housing
Fluorescence and widefield microscopy via ocular	Safety shutter closed automatically	

Operating the MITICO illumination combiner system in **User mode**:

1. Mount a specimen and search for a region of interest using widefield fluorescence illumination. Refer to the manual of the imaging system for detailed descriptions.

2. Be sure to have a filter cube with adequate dichroic mirror in the light path.

3. Close the laser safety stage cover and ocular shutter to enable the opening of the laser safety shutter(s) of the MITICO.

4. Turn on the laser as described in the laser manual. You need to press the **START** buttons of the Olympus Soft Imaging System laser systems to open their safety shutters.

5. Be sure that the light path selector slider in the MITICO illumination combiner is moved to the right in order to open the TIRFM illumination light path (see Chapter 4.8, *The Light Path Selector Slider*).

6. Open the high-speed laser shutter either via software (with the **REMOTE** switch of the LAS-MC manual control set to (**I**) in case TIRF Control.exe is used) or by switching the corresponding **LASER 1**, **2**, **3**, **4** switch of the LAS-MC manual control to open (**I**).

> To protect your specimen from photo-bleaching, close the laser shutter (by reversing step **6**) whenever suitable, even if you interrupt observation only for a short time.

All fiber ports of the cellTIRF MITICO illumination combiner are automatically set to the position they were at when the system was switched off last time. The software control of the MITICO illumination combiner and the adjustment of the beam position are described in detail in Chapter 6, *Using the cellTIRF MITICO Illumination Combiner*.

5.2 The Maintenance Mode

Actions in maintenance mode		
Permitted Action	**Remarks**	**Actions not permitted**
Exchanging microscope or camera hardware	Only with LS shutter closed. Adjustment necessary afterwards.	
Laser adjustment		
Specimen manipulation under TIRF conditions with the stage cover lifted		Executing experiments with remote control of the laser shutters
Specimen observation via ocular under TIRF conditions	Refer to safety precautions above. Only possible with pressed binocular shutter interlock bridge.	
Mounting or exchanging of fiber or laser	Laser shutters must be closed. Line adjustment necessary afterwards.	
Adjustment of laser into the fiber		

For details concerning the setup of the hardware, refer to your hardware manual and the MITICO Illumination Combiner Service Manual.

> Operating in **Maintenance mode** is only allowed to persons authorized to operate and align Class 3B laser products according to the national regulations.
>
> Always wear adequate protection and avoid unnecessary radiation of the laser beam.
>
> While operating the system in maintenance mode, be sure that access to the system is restricted to authorized persons only.

5.2.1 Exchanging Microscope or Camera Hardware

Before exchanging hardware, always consider the compliance of the new hardware to the requirements of TIRF microscopy and the laser equipment. Ask your Olympus specialist for advice in case of uncertainty. The best way to modify a system is with the lasers turned off. After changes affecting the laser light path through the microscope (e.g., changing an objective or a filter cube) it might be necessary to realign the laser fiber ports to have the laser beams exiting the objective vertically when the fiber ports are set to the 0 position; see Chapter 6 *Using the cellTIRF MITICO Illumination Combiner*.

5.2.2 Adjusting the Laser Beam Position

After an exchange of hardware or after moving he system, a new alignment of the laser may be necessary. Only if the laser beam is focused into the back focal plane of the objective and aligned correctly, disturbance by scattered light can be minimized. For details of aligning refer to the cellTIRF Service Manual and the laser manual.

5.2.3 Access to the Specimen without Deactivating the TIRFM System

For some applications it is necessary to manipulate the specimen during an experiment. In **User mode**, however, the laser safety stage cover has to be in place; otherwise the TIRFM system is deactivated because all laser safety shutters remain closed.

Setting the system to **Maintenance mode** by authorized and trained personnel allows removing the laser safety stage cover to gain access to the specimen without causing the laser safety shutters to close automatically. Thus, lifting the laser safety stage cover might cause exposure to laser irradiation if the high-speed shutter of any laser system is open.

Check the position of the laser beam through the translucent laser safety stage cover insert. Be sure to wear adequate laser protection before opening the lid and make sure all high-speed laser shutters are closed. Shield the specimen with a sheet of paper before opening a high-speed laser shutter to make sure that the laser beam does not exit the objective in an unexpected direction.

5.2.4 Observing the Specimen under TIRF Illumination Through the Ocular

Authorized and trained personnel can observe the specimen in **Maintenance mode** via the ocular when taking the safety precautions into account.

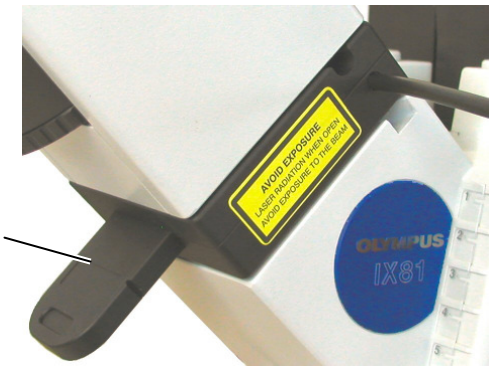
Before observation via the ocular, make sure to have an adequate filter cube that quantitatively blocks the laser light inserted into the light path! The use of an inadequate or damaged dichroic mirror or emission filter can lead to intense, hazardous laser light exiting the ocular. This laser light can irreversibly damage your eyes!

Never change optical elements (filter, dichroic mirror, objective etc.) while observing the TIRF image by eye. The switch of optical elements can lead to reflections of laser light into the ocular.

1. Be sure that the light path selector slider of the MITICO illumination combiner is moved all the way to the right to open the TIRF illumination light path (see Chapter 4.8, *The Light Path Selector Slider*).

2. Activate the **Maintenance mode** by turning the key on the LAS-MC laser manual control to the **MAINTENANCE** position.

3. Open the ocular shutter by pulling the slider out all the way. The laser safety shutter will close automatically.



4. Hold a sheet of paper in front of the eyepieces.

5. Open the laser safety shutters of MITICO illumination combiner and lasers by pressing the red ocular safety interlock bridge button of the LAS-MC manual control. Check the sheet of paper to control the light intensity emitting from the ocular pieces before looking through the eyepiece.

Control or adjustment procedures other than those described will lead to hazardous laser beam exposure!

6 Using the cell^tirf MITICO Illumination Combiner

The cell^tirf illuminator system and the Olympus Soft Imaging Solutions laser systems are fully integrated in the cell^M / cell^R imaging software. In case the cell^tirf illuminator system is to be used within an external system the cell^tirf Control stand-alone software is provided to operate the MITICO Illumination Combiner via the AD-USB-ODB adaptor from any PC. This chapter describes the operation of the MITICO by both cell^M / cell^R and cell^TIRF Control software.

6.1 The cell^TIRF Control Software....32
6.2 cell^tirf MITICO Control via the cell^R Software....38
6.2.1 System Configuration....38
6.2.2 Operating cell^tirf MITICO....39
6.2.3 TIRFM in the Experiment Manager....41

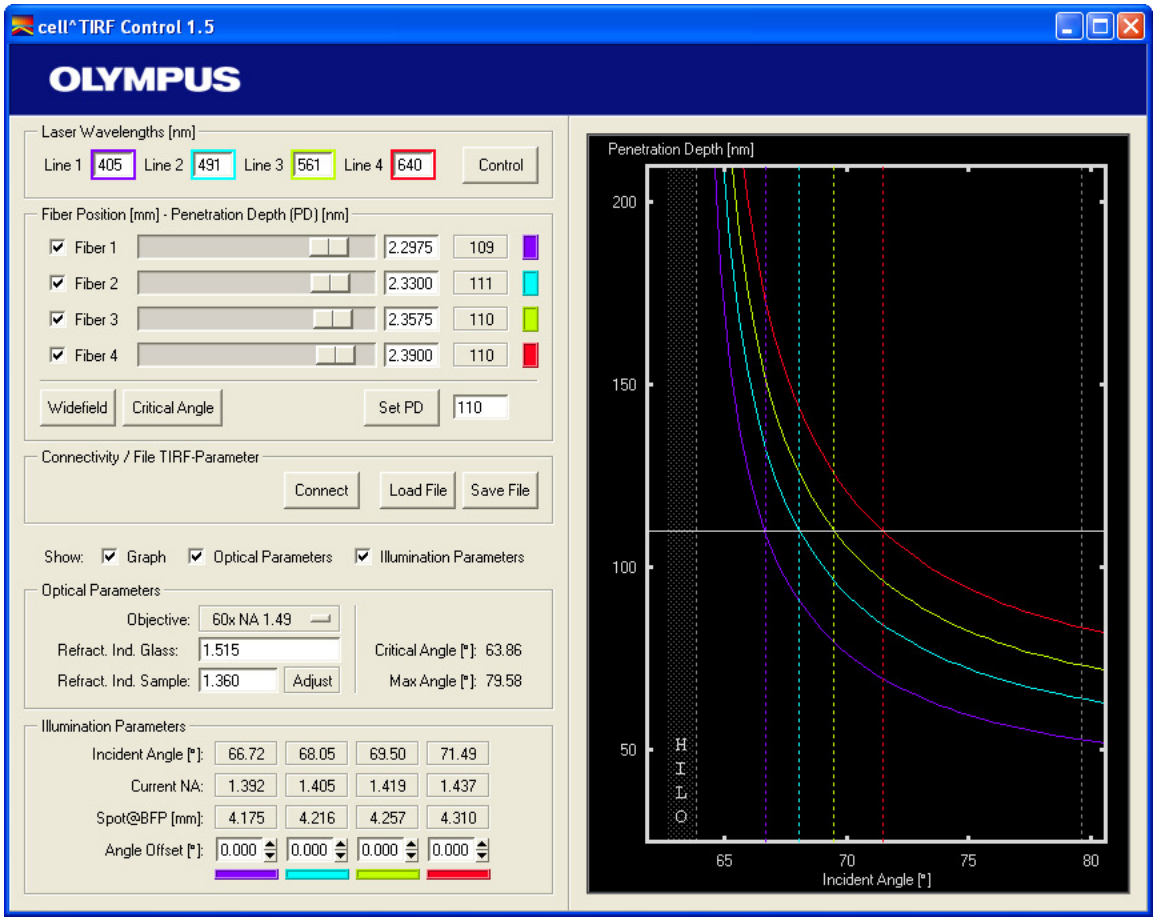
6.1 The cell^TIRF Control Software

cell^TIRF.exe icon

Execute the **cell^TIRF.exe** file to start the software.

The first step is to click the **Connect** button to establish the communication between PC and the cell^TIRF MITICO illumination combiner. See below for detail.

The control window contains two parts. On the left is a graphical display of the dependency of beam incident angle and penetration depth of the evanescent field. On the right are the control sliders for the four laser ports and the parameter settings of the optical system.



Laser Wavelengths [nm]

Make sure that the lasers are connected to the fiber ports in the right order. Connect the shortest wavelength laser to the upmost port and the other lasers in the order of increasing wavelength; for

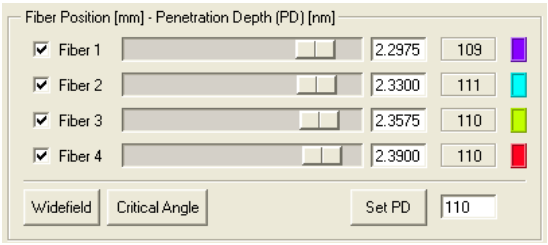
example, in a typical four laser system a 405 nm laser must be connected to the top port, a 640 nm laser to the lowest port and a 488 nm and a 561 nm laser correspondingly to the second and third ports from top.

Line 1. Set the wavelength of the first laser. This is the one connected to the upmost fiber port and is the one with the shortest wavelength.

Line 2, **Line 3**, **Line 4**. Set the wavelengths of the other lasers in the order of increasing wavelength.

Control. Click this button to open the **Laser Control** window. See the description at the end of this chapter.

Fiber Position [mm] – Penetration Depth (PD) [nm]



The sliders. Move a slider by mouse drag to move the corresponding fiber to a certain position. Activate a slider via mouse click and you can move the slider step by step by using the mouse scroll wheel.

Fiber Position [mm] edit controls. The given value corresponds to the slider position and gives the current position of the fiber relative to the zero position. You can set it also by typing in a value between 0 and about 2.8. The maximum value will be set automatically if the number typed is higher than can be reached by the system.

Penetration Depth (PD) [nm]. This is the value calculated from the current settings. See the **Graph** description below for the definition of PD.

Widefield button. Click here to move all checked **Fibers** to the zero position.

Set Penetration Depth button. Click here to move all checked **Fibers** to the desired PD value.

Set Penetration Depth. Type the desired PD value.

Check boxes. Check here to cause a fiber port to move upon clicking the **Widefield** and **Set Penetration Depth** buttons. All other fibers will remain in their current positions.

Connectivity / File TIRF Parameters

Connect button. Click here to initialize communication between the AD-USB-ODB adaptor and the MITICO illumination combiner.

Save File button. Click here to store the current parameters in a file.

Load File button. Click here to load a parameter file.

Show:
The following check boxes activate/deactivate the display of features of the **cell^TIRF Control** window.

The graph
It shows a plot of penetration depth vs. incident angle for each wavelength in the respective color.

Graph. Check/uncheck this box to display/hide the graph.

Penetration Depth [nm]. The penetration depth of the evanescent field is defined as the distance from the cover slip-to-water interface where the electromagnetic field of the totally reflected laser beam is decayed to 1/e. It is wavelength dependent.

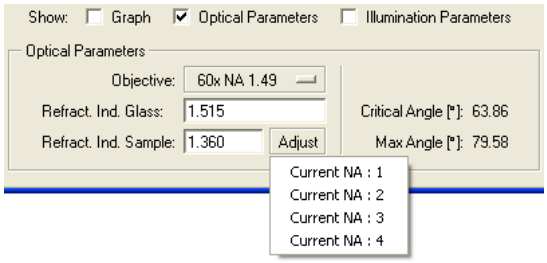
Incident Angle [°]. This is the angle of the laser beam relative to the normal of the cover slip-to-water interface. It is directly related to the position of the beam relative to the optical axis, i.e., to the offset of the beam relative to the center of the back aperture of the objective.

Dotted vertical lines. They indicate the current incident angle of each laser beam.

Horizontal white line. It indicates the current value in **Set Penetration Depth**.

HILO. The acronym stands for Highly Inclined and Laminated Optical sheet microscopy. The dotted, white vertical line is at the position of the critical angle. In the HILO range, just before TIRF illumination starts, the laser beam enters the medium in a very shallow angle. Thus the bulk of the sample is not being illuminated and an increased Z-resolution as compared to the widefield illumination is achieved.

Optical Parameters
These parameters serve to calculate the theoretical values of critical angle, penetration depth and so on.



Optical Parameters. Check/uncheck this box to display/hide these parameters

Objective. Select the objective in use from the shortlist. It contains all Olympus objectives dedicated for TIRF applications as well as some further objectives with a sufficiently high NA.

Refract. Ind. Glass. Set the value as given by the manufacturer.

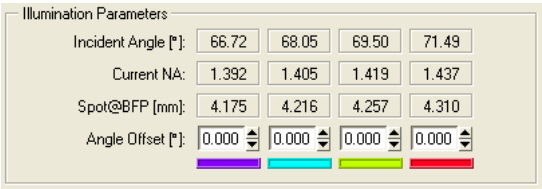
Refract. Ind. Sample. Set the estimated value of the sample in use. Typical values are 1.36 – 1.38.

Adjust. You may correct the set refractive index of the sample by reading in the **Current NA** of one of the laser lines. The refractive index of the sample equals the **Current NA** if the system is carefully aligned and a laser line is set empirically (!) to the **Critical Angle**, .i.e., this approach requires the line to be set to the transition point between widefield and TIRF illumination.

Critical Angle [°]. This is the critical angle as calculated from the settings.

Max. Angle [°]. This is the calculated maximum angle for the active wavelength that can be obtained with the objective in use in dependency of the current settings. This corresponds to the position where the beam reaches the outmost periphery of the objective back aperture.

Illumination Parameters



Show Illumination Parameters. Check/uncheck this box to display/hide additional, calculated, optical parameters.

Incident Angle [°]. This is the angle of the laser beam relative to the normal of the glass-to-medium interface. settings. See the **Graph** description above for the definition of the **Incident Angle**.

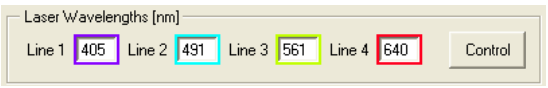
Current NA. This parameter relates the **Incident Angle** to the NA of the objective. In the widefield position, i.e., if the beam is on the optical axis the NA is 0. TIRF starts when the **Current NA** equals the refractive index of the medium and ends when the **Current NA** equals the NA of the objective.

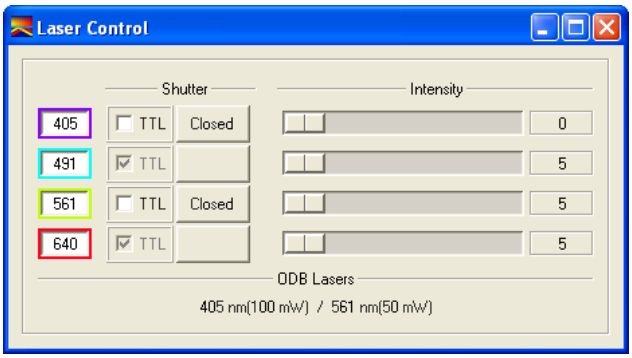
Spot@BFP [mm]. This is the distance of the laser beam from the optical axis at the objective back focal plane.

Angle Offset [°]. These controls enable you to adjust the system in case a laser line is empirically not at the critical angle, i.e., in the transition between widefield and TIRF illumination, even if the line is set to the calculated critical angle (by clicking on the **Critical Angle** button). See a detailed description at the end of this chapter.

The Laser Control Window

Control. Click this button to open the **Laser Control** window.





Shutter: TTL check box. Activate this if the laser shutter is to opened/closed via a TTL signal though the LAS-BOB breakout box; see Chapter 4.4, *The LAS-BOB Laser Safety Break-out Box*. Do NOT check this box in case Olympus Soft Imaging Solutions lasers are connected via the ODB device bus.

Shutter: Shutter button. It is active only for Olympus Soft Imaging Solutions lasers if **TTL** is NOT activated. Click here to open/close the laser shutter.

Intensity sliders. They are active only for Olympus Soft Imaging Solutions lasers connected via the ODB device bus. Use the slider to adjust the intensity of the laser.

ODB Lasers. This field lists all Olympus Soft Imaging Solutions lasers connected via the ODB device bus.

> You can use the **Illumination Parameters** to check if your assumption of the refractive index of the medium is correct:

1. Move the fiber to the position where the **Current NA** equals the refractive index of the medium.

2. Set the system to **Maintenance Mode** using the key on the LAS-MC manual control; see Chapter 5.2, *The Maintenance Mode*.

3. Lift the laser safety stage cover.

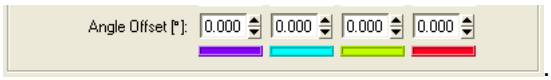
4. Move the fiber slightly back and forth to find the position where the laser beam just disappears.

5. Adjust the **Refract. Medium** value if necessary.

> There are several possible reasons for not reaching TIRF illumination when the **Current NA** equals the given **Refract. Medium** value.
> – Either the estimation of the refractive index was not correct
> – or the fiber alignment is not perfect, i.e., the beam in the **Widefield** position does not exit the objective on the optical axis (vertically)
> – or the sample is slightly tilted.
>
> The **Angle Offset** function (in the **Illumination Parameters** field) allow to correct for such a mismatch between theory and praxis if neither the alignment can be improved nor a sample tilt corrected or if the assumption of the refractive index of the sample (**Refract. Ind. Sample**) shall not be adjusted.

1. Move the laser beam to the **Critical Angle.**

2. Change the position of the laser port by using the arrow buttons Use the **Angle Offset** arrow buttons (NOT the fiber position slider) until the critical angle is reached.



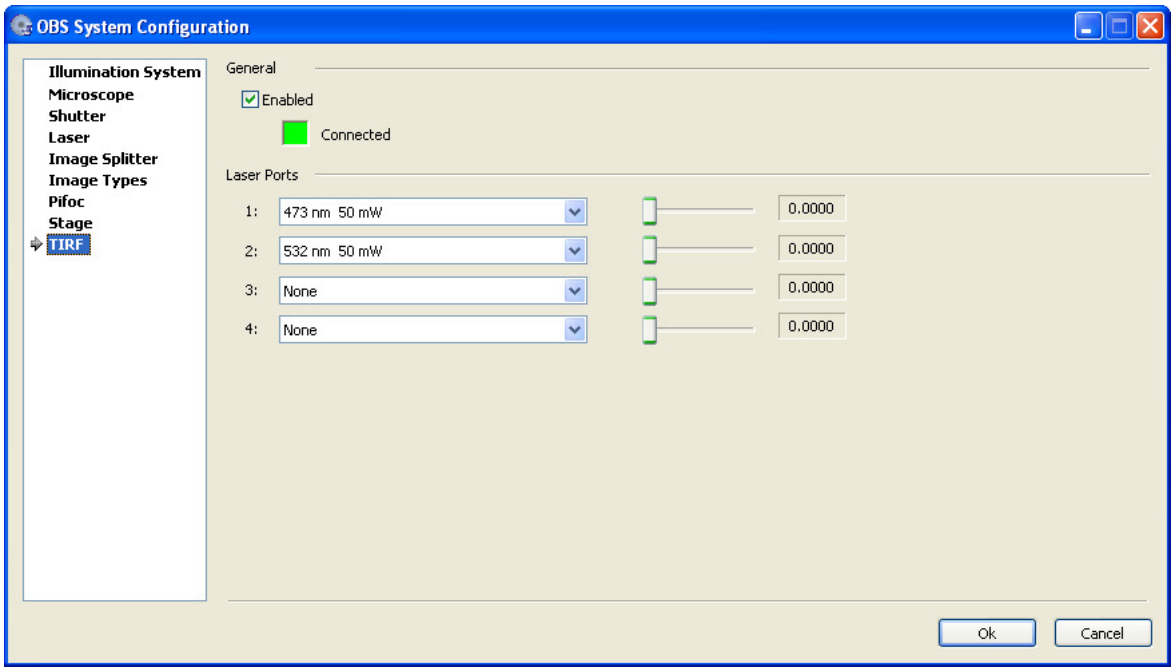
6.2 cell^tirf MITICO Control via the cell^R Software

6.2.1 System Configuration

1. Start the ObsConfig software to configure the excitation filters

2. Make sure that the lasers and corresponding image types are configured on pages **Microscope ▸ Laser** and **Microscope ▸ Image Types** as described in the laser manual.

3. Go to page **Microscope ▸ TIRF**.
   In case this page is not available right-click on OBS System Configuration (top left), open the **Components** context menu and activate **TIRF**.



4. Click **Enabled** to activate the MITICO illumination combiner.

5. For each **Laser Port** select the correct laser from the shortlist. Mind that the lasers are to be connected to the MITICO laser ports in the order of increasing wavelength.

6. Exit the software with **OK**.

6.2.2 Operating cell^tirf MITICO

Optical Parameters

These parameters serve to calculate the theoretical values of critical angle, penetration depth and so on.

Objective. Select the objective in use from the shortlist. It contains all Olympus objectives dedicated for TIRF applications as well as some further objectives with a sufficiently high NA.

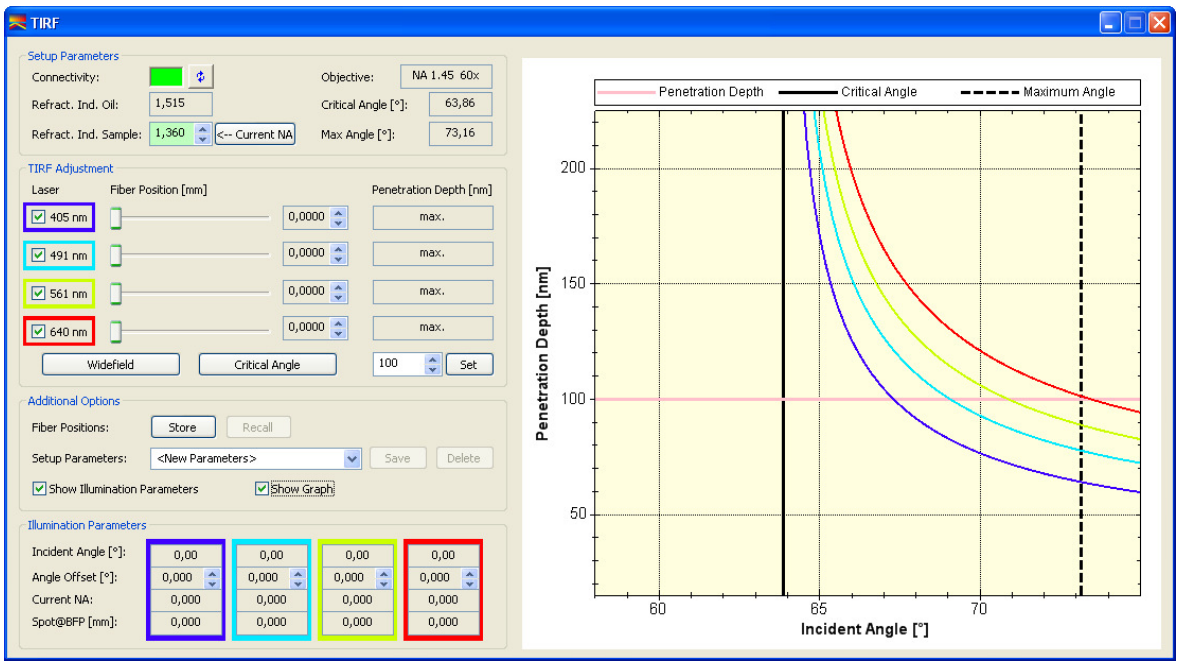
Refract. Ind. Oil. Set the value as given by the manufacturer.

Refract. Ind. Sample. Set the estimated value of the sample in use. Typical values are 1.36 – 1.38.

← **Current NA** button. Click here to read in the **Current NA** value. See below for details.

Critical Angle [°]. This is the critical angle as calculated from the set **Optical Parameters**.

Max. Angle [°]. This is the maximum angle for the active wavelength as calculated from the set **Optical Parameters** that can be obtained with the objective in use. This corresponds to the position where the beam reaches the outmost periphery of the objective back aperture.



TIRF Adjustment

Laser check boxes. Check here to cause a fiber port to move upon clicking the **Widefield**, **Critical Angle** and **Set Penetration Depth** buttons. All other fibers will remain in their current positions.

Fiber Position [mm] sliders. Move a slider by mouse drag to move the corresponding fiber to a certain position. Activate a slider via mouse click and you can move the slider step by step by using the mouse scroll wheel.

Fiber Position [mm] edit controls. The given value corresponds to the slider position and gives the current position of the fiber relative to the zero position. You can change it by typing in a value between

0 and about 2.8. The maximum value will be set automatically if the number typed is higher than can be reached by the system.

Penetration Depth [nm]. These are the value calculated from the current settings. See below for the definition of PD.

Penetration Depth [nm], Edit Control. Type the desired PD value or change it by using the up/down arrow buttons.

Penetration Depth [nm], **Set** button. Click here to move all checked **Laser** fibers to the set PD value.

Widefield button. Click here to move all checked **Laser** fibers to the zero position.

Critical Angle button. Click here to move all checked **Laser** fibers to the calculated critical angle position.

There are several possible reasons for TIRF not starting when the **Current NA** equals the given **Refract. Ind. Sample** value.
– Either the estimation of the refractive index of the sample is not correct
– or the fiber alignment is not perfect, i.e., the beam in the **Widefield** position does not exit the objective on the optical axis (vertically)
– or the sample is slightly tilted.
The **Fiber Offset** function and the resulting **Offset Inc. Angle [°]** (see below) serve to optimize the settings.

Additional options

Fiber Positions Store button. Click here to temporarily store the current fiber positions. This allows you to quickly **Recall** them when "playing around" with the different fiber ports.

Fiber Positions Recall. Click here to recall the temporarily stored fiber positions.

Setup Parameters shortlist. Select a saved set from the list to load it. Type in a new name and store a new entry via the **Save** button.

Setup Parameters Save button. Click here to save the current settings.

Setup Parameters Delete button. Click here to delete the active entry from the shortlist.

Illumination Parameters

Show Illumination Parameters. Check this option to show additional, calculated, optical parameters.

Incident Angle [°]. This is the angle of the laser beam relative to the normal of the glass-to-medium interface.

Current NA. This parameter relates the **Incident Angle** to the NA of the objective. In the widefield position, i.e., if the beam is on the optical axis the NA is 0. TIRF starts when the **Current NA** equals the refractive index of the medium and ends when the **Current NA** equals the NA of the objective.

Spot@BFP [mm]. This is the distance of the laser beam from the optical axis at the objective back focal plane.

Fiber Offset. You may observe that the laser beam has not yet reached the critical angle or is already beyond it when the **Current NA** equals the theoretical **Critical Angle**. You may correct the settings by

changing the **Refract. Ind. Sample** value. If you prefer, however, not to change the refractive index of the sample you can fine adjust the system in the following way:

1. Click the **Critical Angle** button to move the laser port to the position were the beam would theoretically reach the critical angle.

2. Set the system to **Maintenance Mode** using the key on the LAS-MC manual control; see Chapter 5.2, *The Maintenance Mode*.

3. Lift the laser safety stage cover.

4. Use the **Fiber Offset** up/down arrow buttons to move the fiber port slightly back and forth to find the position where the laser beam just disappears.

5. Close the stage cover.

The system will now always add the **Fiber Offset** to the set **Fiber Position**.

Offset Inc. Angle [°]. This is the incident angle offset caused by the **fiber offset**.

The graph.
It shows a plot of penetration depth vs. incident angle for each wavelength in the respective color.

Show Graph. Check this option to show a graphical display of the dependency of beam incident angle and penetration depth of the evanescent field.

Dotted vertical lines. They indicate the current incident angle of each laser beam. The colors correspond to the laser wavelengths.

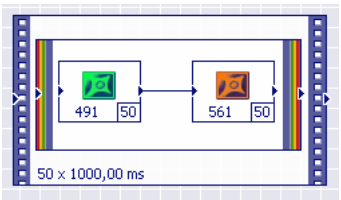
Horizontal magenta line. It indicates the current value in **Set Penetration Depth**.

Critical Angle. It is indicated by the vertical black line.

Maximum Angle. It is indicated by the dotted vertical black line.

6.2.3 TIRFM in the Experiment Manager

Prior to starting a standard experiment the different laser lines are usually set by the user to positions that correspond to a certain penetration depth of the evanescent field. Thus, during such an experiment the cell^TIRF illuminator is just passively guiding the laser beams and its motorization is not being used. Consequently it does not appear in the experiment plan. The Experiment Plan of course needs to contain the Image Acquisition commands that make use of the different lasers as light sources.



It is, however, possible to control the beam position during the experiment in order to change penetration depth or switch from TIRF to widefield illumination, for example.

The corresponding **TIRF** command icon is placed on the **Lightsource Commands** toolbar in the Experiment Manager. If not visible activate it via **View ▸ Lightsource Commands**. Its properties page lists three parameters for each laser line.

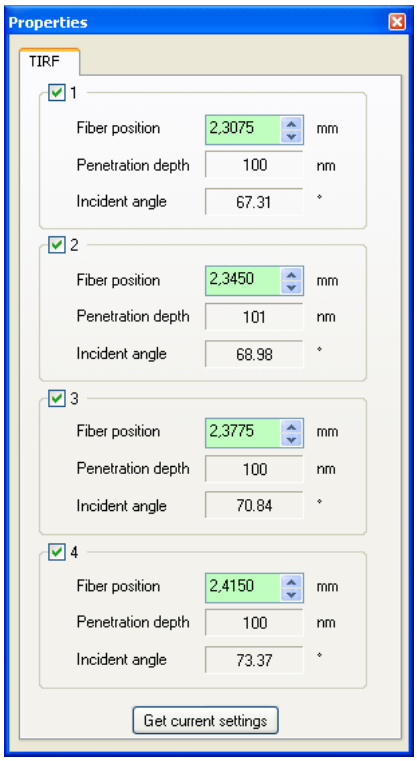
Properties

Check box. Click here to activate a motorized fiber port. If disabled, the position will not change upon execution of this command during the experiment.

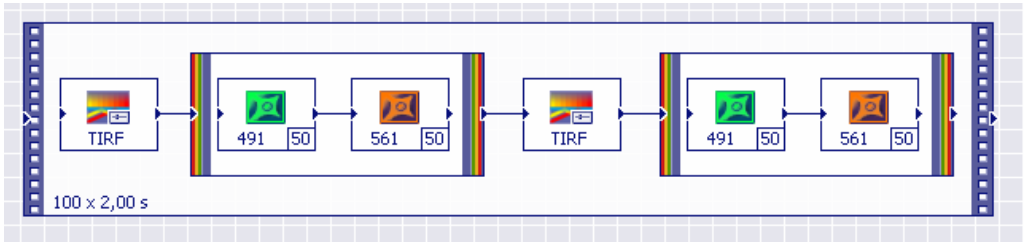
Fiber position. Type in a value or use the arrow buttons to increase or decrease the value.

Penetration depth, Incident angle. The displayed values are automatically calculated based on the **Fiber position** and the **Setup Parameters** in the **TIRF** control window, including the selected objective.

Get current settings. Click here to set each **Fiber position** to the current one (according to the **TIRF** control window).



In an Experiment Plan the **TIRF** command icon can be placed where ever suitable, for example before **Acquisition** commands, **Multicolor** loops or **Time-lapse** loops:



7 Specifications and Technical Data

Specifications and Technical Data

Dimensions and Weight (MITICO): H 182 x W 420 x D 282 mm (incl. exit optics tube and flange: 154 mm); 7 kg

Power supply rating: 24 V, 1.88 A, DC power supply connected to LAS-BOB

Enclosure rating: IP20

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 5with IEC664)

Compatible microscope frames: IX2 series (IX81 / IX71 / IX51)

Fiber ports: FCP8 (FCP possible), 4-axis alignment

Fiber port motorization, absolute movement range: 3 mm, repositioning precision >99.9%

Fiber port motorization, full range move: < 500 ms

Widefield illumination port: fits standard lamp housing (e.g., U-LH100HGAPO), filter wheels (e.g., U-FFWO, U-FWO), shutters (e.g., U-FSHA, IX2-SHA) or fiber illuminator TIRMT-CON

Widefield transmission: 340 – 800 nm

Throughput efficiency @ line 1 via mirror: > 25% (typically > 35%)

Illumination selector slider: either 100% widefield illumination or 50% widefield illumination combined with TIRF illumination

Field iris diaphragms: diameter adjustable between 1.7 and 13 mm

Laser beam combining dichroic mirrors: reflect p-polarized laser beam

Polarizing beam splitter combining lasers beams and widefield illumination: reflects s-polarized laser beam, transmit p-polarized widefield illumination light

Laser clean-up filter sliders: for 25 mm diameter filters

FRAP lens, line 1 only: moves laser focus from back focal plane to focal plane

Laser safety certified for: 400 – 700 nm, total input power 500 mW

Laser safety features:

- Ocular shutter with interlock
- Stage cover with interlock and translucent PLEXIGLAS SATINICE ® DC, color: carbon, sort: 7C17 DC, thickness: 6 mm
- Two (redundant) laser safety shutters inside MITICO illuminator
- Interlock key switch
- Laser safety shutters "OPEN" indicator diodes
- Emergency interrupter

OLYMPUS®

OLYMPUS EUROPA HOLDING GMBH
Postfach 10 49 08, 20034, Hamburg, Germany

OLYMPUS AMERICA INC.
3500 Corporate Parkway, Center Valley, PA 18034, USA

OLYMPUS AUSTRALIA PTY. LTD.
31 Gilby Road, Mt. Waverley, VIC 3149, Melbourne, Australia

OLYMPUS LATIN AMERICA, INC.
6100 Blue Lagoon Drive, Suite 390 Miami, FL 33126-2087, USA

OLYMPUS SINGAPORE PTE LTD.
491B River Valley Road, #12-01/04 Valley Point Office Tower, Singapore 248373

Printed in Germany 2010 09 M 003