

## INSTRUCTIONS

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**cell**<sup>frap</sup>

### SOFTWARE MODULE AND LASER SAFETY FOR THE HIGH-SPEED SCANNING FRAP SYSTEM

This instruction manual describes the configuration and usage of the **cell**<sup>frap</sup> scanning FRAP system within the Olympus **xcellence** Imaging Stations. To ensure safety, obtain optimum performance and familiarize yourself fully with the use of the product, we recommend that you study this manual thoroughly before operation. Together with this manual, please also read the **cell**<sup>frap</sup> hardware manual, the **xcellence** hardware and software manuals as well as the instruction manuals of the microscope in order to understand overall operation methods. Retain this manual in an easily accessible place near a system for future reference.

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# Imaging Excellence

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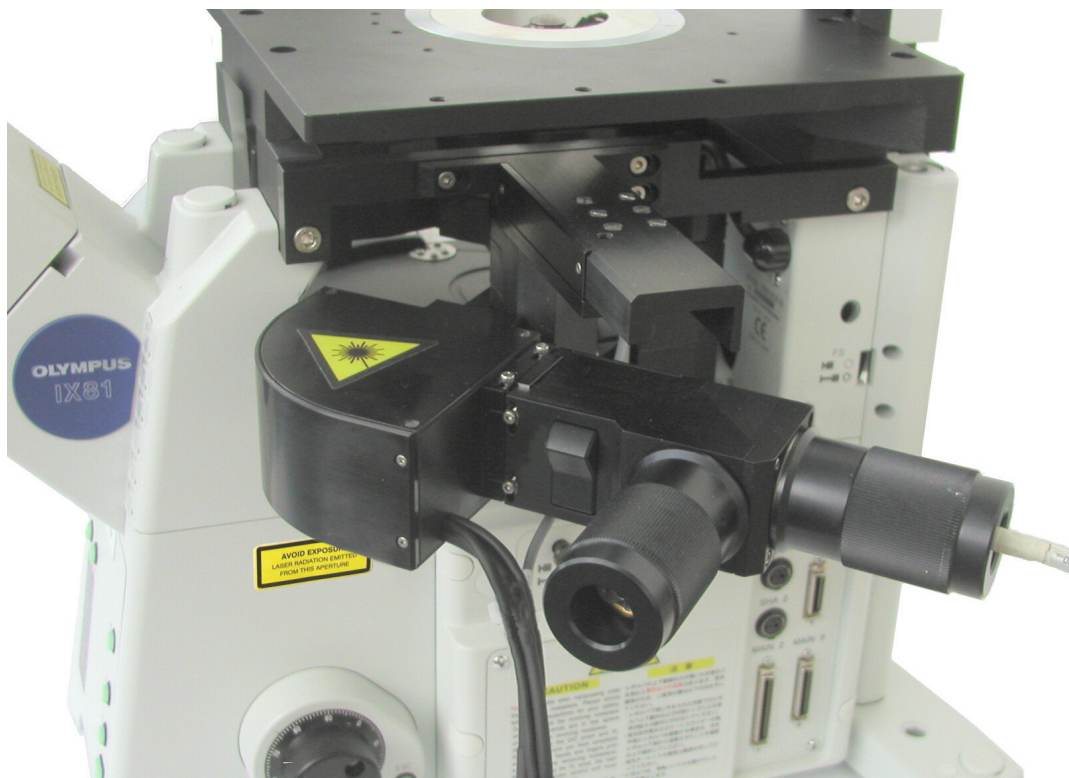
# 1 Introduction

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Thank you very much for purchasing Olympus Soft Imaging Solutions' state of art **cell<sup>frap</sup>** scanning FRAP 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 **cell<sup>frap</sup>** scanning FRAP system is designed for general laboratory use only. It allows coupling up to two lasers to an inverted Olympus IX2 FRAP microscope.

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cell<sup>FRAP</sup> scan head mounted on an IX81

## 1.1 Abstract

In the following sections you will find a detailed description of the components of cell<sup>FRAP</sup> scanning FRAP system, handling and alignment instructions and how to maintain the performance of your FRAP microscope. Keep in mind that all components are designed to work together with an Olympus xcellence Imaging Station 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 microscope frames IX81S1F-FRAP, IX81S1F-FRAP-ZDC, and IX71S1F-FRAP, as well as a range of diode laser systems between 405 nm and 700 nm 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 500 mW.

This instructions manual describes the hardware components of the cell<sup>frap</sup> scanning FRAP system, and their installation. It also explains the software configuration and the use o the system.

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<sup>frap</sup> scanning FRAP system. Keep this manual close to the imaging station for future reference.

The cell<sup>frap</sup> scanning FRAP system meets the CE requirements:

- EN61326 Class A
- EN60825

## 1.3 Components

The cell<sup>frap</sup> scanning FRAP system consists of the following components:

- a Laser shutter hand switch LAS-MC with key with emergency interrupter and key-controlled switch to maintenance mode
- b Laser safety break-out box LAS-BOB
- c Laser safety stage cover with translucent laser safety glass that allows following the beam position
- d Laser safety ocular shutter
- e Manual

The cell<sup>frap</sup> software module for the *xcellence* imaging software is likewise required as well as at least one laser, favorably a dedicated OSIS FRAP diode laser:

Furthermore the following scanner head and controller shall be used (Rapp Optoelectronic):




- a **cell**<sup>fire</sup> illuminator featuring two fiber ports, each with adjustable line focus
- b **cell**<sup>fire</sup> control unit

## 2 Safety Symbols





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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 cell<sup>FRAP</sup> scanning FRAP 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 **cell<sup>frap</sup>** scanning FRAP 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.
	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.	<b>cell<sup>frap</sup></b> scan head
	Laser radiation can cause irreversible damage to your eyes. Wear adequate protection before opening this aperture.	Microscope frame, right side, left side near C-mount; see photos <b>2</b> and <b>3</b> .
	These apertures should be closed while operating the system. Parts connected should be dismantled by trained personal only and when the laser is turned off.	Laser safety ocular shutter; see photo <b>4</b> .
	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 <b>5</b> .

Labels for the microscope components are included in the shipping list of the Olympus **cell<sup>frap</sup>** scanning FRAP 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<sup>frap</sup> scan head



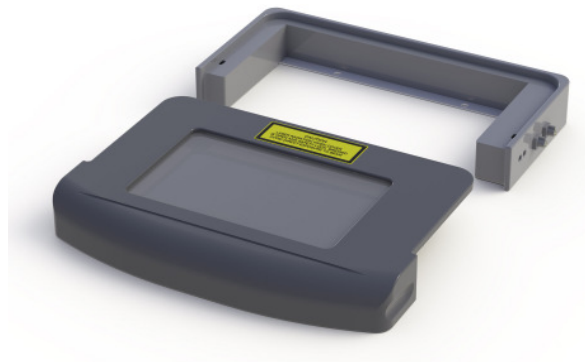
2: microscope frame, right side



3: fluorescence turret and C-mount



4: laser safety ocular shutter



5: laser safety stage cover



## 3 Safety Precautions and Handling Instructions

The Olympus cell<sup>frap</sup> scanning FRAP 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

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## 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 **cell<sup>FRAP</sup>** scanning FRAP system check if all devices are attached correctly and all apertures are closed.

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

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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.

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4. Always operate the **cell<sup>FRAP</sup>** scanning FRAP 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.

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

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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.

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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.

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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 cell<sup>laser</sup> scanning FRAP 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!

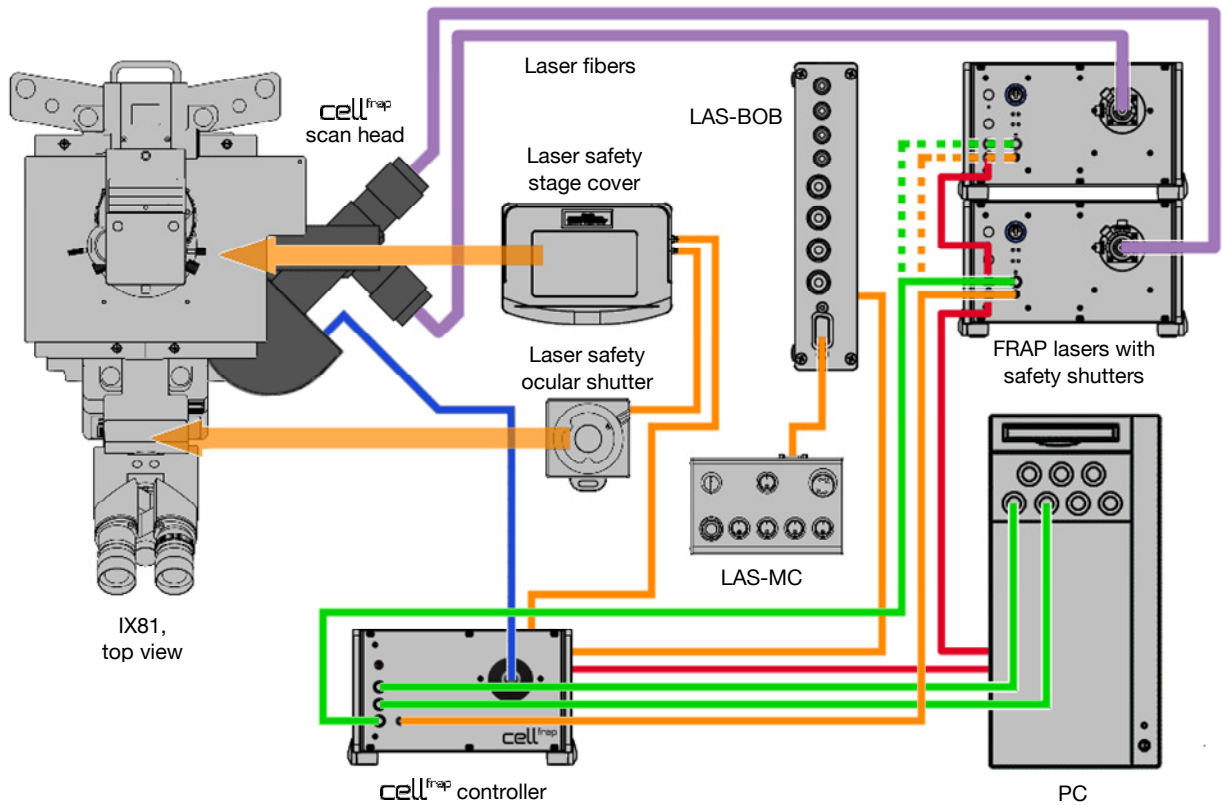


## 4 cell<sup>frap</sup> Overview and Laser Safety Setup

The following chapter describes all components and interfaces of the Olympus cell<sup>frap</sup> scanning FRAP system including all laser safety devices and how they are installed and connected.

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## 4.1 Setup Scheme



Scheme of an IX81 microscope with a **cell<sup>frap</sup>** scanning FRAP system, two lasers and the laser safety equipment.

- Connections of the laser safety devices
- ODB / RS232 communication connections
- TTL trigger connections
- FRAP scanner communication connection
- Laser fibers

## 4.2 The Laser Safety Features – Overview and Check

The laser safety concept of the cell<sup>frap</sup> scanning FRAP system consists of the following modules:

- 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
- A double-redundant laser safety shutter in front of the exit optics of the Olympus Soft Imaging Solutions lasers

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.

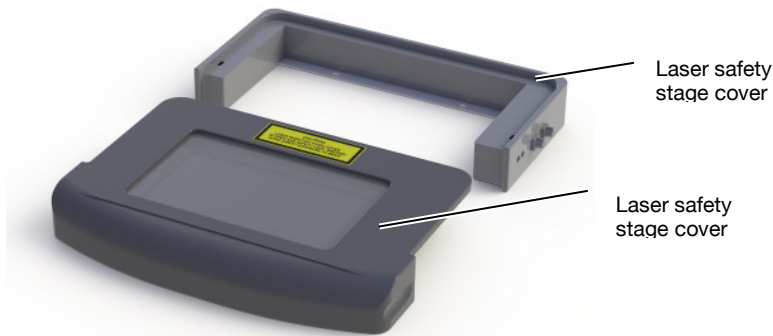


Laser safety ocular shutter

- 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 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 one of the 5-pin **SAFETY INTERLOCK** plugs at the back panel of the **cell<sup>frap</sup>** control unit.
- The other 5-pin **SAFETY INTERLOCK** plug at the back panel of the **cell<sup>frap</sup>** control unit must be connected to the 5-pin **STAGE COVER** plug of the LAS-BOB laser safety breakout box.

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

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

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2. Switch on the FRAP laser via the software; see Chapter 6.3.1, *The FRAP GUI in xcellence*. You should observe laser light through the translucent stage cover insert.

---

3. 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.

---

4. 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

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

**i** 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 Mounting the Laser Safety Kit

1. Remove the ocular from the microscope frame and mount the ocular laser safety shutter onto the ocular tube (with the cable on the right hand side) and fix it as shown in the photo below.

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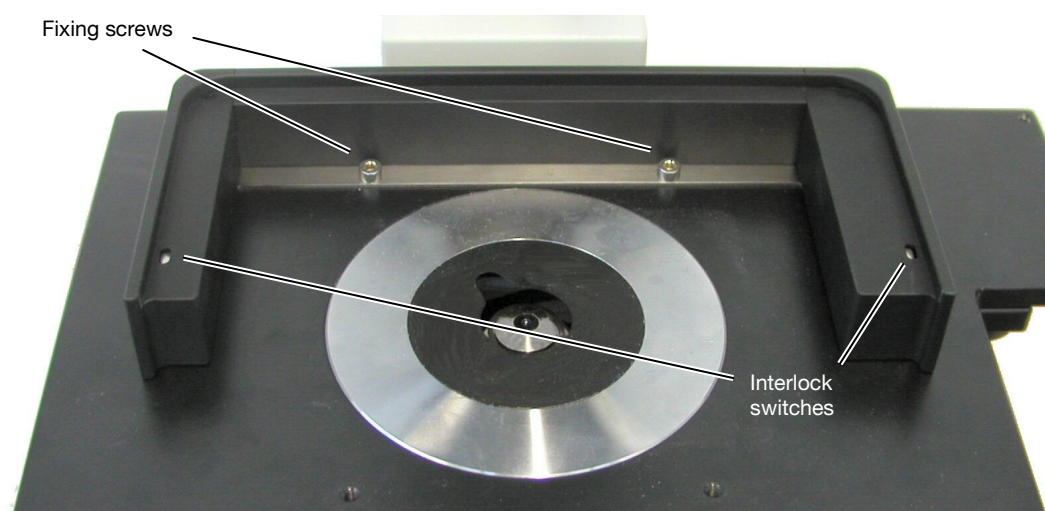
2. Mount the ocular back on top of the ocular laser safety shutter and tighten the holding screw.

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3. Mount the U-shaped frame of the laser safety stage cover with the connector plugs pointing to the right onto the microscope stage fixing it with the two M4x5 fixing screws.



4. Place the lid of the laser safety stage cover on top of the stage frame so that the two pins on the lid fit into the two holes of the frame that host the interlock switches.



5. Plug the cable of the laser safety ocular shutter with one of the plugs of the laser safety stage cover.

6. Connect the second plug of the laser safety stage cover with the **5-pin female STAGE COVER** plug of the **cell<sup>frap</sup>** scanning illuminator using the corresponding cable.



## 4.4 Connecting the **cell<sup>frap</sup>** Control Unit

The installation of the **cell<sup>frap</sup>** scanning illuminator is described in detail in the hardware manual. Only the connection of the cables of the **cell<sup>frap</sup>** scanning illuminator and the control unit shall be repeated here. These steps are partly repeated in the following sub-chapters as well.



1. Connect the **5-pin female STAGE COVER** plug at the LAS-BOB back panel with one of the 5-pin **SAFETY INTERLOCK** plugs at the back panel of the **cell<sup>frap</sup>** control unit using the corresponding cable.
2. Connect the other 5-pin **SAFETY INTERLOCK** plug at the back panel of the **cell<sup>frap</sup>** control unit with one of the plugs of the laser safety stage cover (and make sure that the second one is connected to the laser safety ocular shutter).

3. Connect the **PC RS232** plug at the back panel of the cell<sup>frap</sup> control unit with a serial port of the system PC.

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4. Connect the cable coming from the scan head with the multi-pin plug at the front panel of the cell<sup>frap</sup> control unit.

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5. Connect the **TRIGGER IN** TTL BNC plug of the cell<sup>frap</sup> control unit with the DIG I/O plug at the imaging computer front panel that is configured as TTL OUT port in the software; see Chapter 6.1, *System Configuration*.

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6. Connect the **TRIGGER OUT** TTL BNC plug of the cell<sup>frap</sup> control unit with the DIG I/O plug at the imaging computer front panel that is configured as TTL IN port in the software; see Chapter 6.1, *System Configuration*.

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7. Connect the **LASER** TTL BNC plug of the cell<sup>frap</sup> control unit with the **SHUTTER** TTL BNC plug of the FRAP laser.

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8. Connect the **INTERLOCK OUT** plug of the cell<sup>frap</sup> control unit with the **INTERLOCK** plug at the front panel of an Olympus laser dedicated for FRAP usage using the Cinch cable provided with it. Connect any other laser in an analogously.

## 4.5 Connecting the LAS-BOB Laser Safety Break-out Box

1. Connect the female **LAS-MC** D-sub 15 plug at the LAS-BOB front panel with the female D-sub 15 plug of the LAS-MC laser manual control using the corresponding male-male cable.



2. Connect the 5-pin female **STAGE COVER** plug at the LAS-BOB back panel with one of the 5-pin female **SAFETY INTERLOCK** plugs at the back panel of the cell<sup>frap</sup> control unit using the corresponding cable. Make sure the cell<sup>frap</sup> control unit is connected with the laser safety stage cover and the laser safety ocular shutter.



3. Connect the 24 V  $\text{---} \ominus \oplus$  DC power supply plug with the 24 V DC power supply.

4. Connect the power supply with a power outlet.

The LAS-BOB additionally features on the front panel:

- Four **INTERLOCK 4 – 3 – 2 – 1** Cinch plugs. These plugs are without function in a cell<sup>frap</sup> system.
- Four **SHUTTER 4 – 3 – 2 – 1** BNC TTL-OUT plugs. These plugs are without function in a cell<sup>frap</sup> system.

The LAS-BOB additionally features on the back panel:

- Two **SAFETY SHUTTER** 4-pin female plugs. These plugs are without function in a cell<sup>frap</sup> system.
- Four **DIGITAL I/O 1 – 2 – 3 – 4** BNC TTL-IN. These plugs are without function in a cell<sup>frap</sup> system.

## 4.6 The Laser Shutter Manual Control

Connect the female **LAS-MC** D-sub 15 plug at the LAS-BOB front panel with the female D-sub 15 plug of the LAS-MC laser manual control using the corresponding male-male cable; see previous chapter.

**i** Note that the LAS-MC manual control cannot be used to open / close the shutter of the FRAP laser because its TTL In plug is connected to the FRAP controller and not the LAS-BOB.

The laser shutter manual control features additionally:

- Four **LASER 1, 2, 3, 4** switches. These plugs are without function in a cell<sup>frap</sup> system.
- A **REMOTE** switch to set the laser shutters to remote control via external TTL triggers or ODB device bus communication (typically controlled by a software like **xcellence**). This switch is without function in a cell<sup>frap</sup> system.

- 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 closes 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.



## 4.7 Key Control

The LAS-MC laser manual control has a lock. When the key is removed or when the lock is set to **User** mode, lifting the laser safety stage cover or opening the laser safety ocular shutter causes all laser safety shutters to close.

When set to **Maintenance** mode, the laser safety stage cover can be lifted and the laser safety ocular shutter can be opened – when the red button is additionally kept pressed – without causing the laser safety shutters to close.

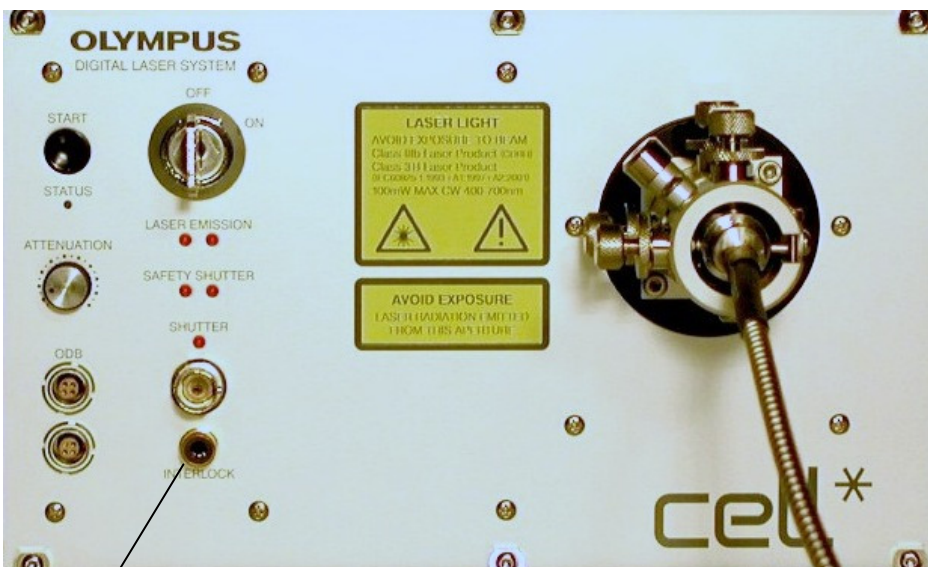


The cell<sup>frap</sup> system is a Laser Safety Class 3B product and may be operated in **Maintenance** mode by trained and authorized personnel only with the trained laser safety officer being informed.

Upon leaving the system, the person in charge has to set the LAS-MC laser manual control to **User** mode and remove the key to protect the system against unauthorized use.

## 4.8 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 of the dedicated FRAP laser must be connected to the **INTERLOCK OUT** plug at the front panel of the **cell<sup>frap</sup>** control via a Cinch cable.



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.

In order to comply with laser safety regulations the interlock connectors of non-Olympus Soft Imaging Solutions laser systems must be connected in the same way.

## 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 cell<sup>frap</sup> scanning FRAP system. Only trained personnel are allowed to operate the system in maintenance mode.

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## 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 <sup>FRAP</sup> / cell <sup>AR</sup> 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 cell<sup>FRAP</sup> scanning FRAP 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.

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

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3. Close the laser safety stage cover and ocular shutter to enable the opening of the laser safety shutter(s) of the Olympus Soft Imaging System laser systems.

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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. Open the high-speed laser shutter via software; see Chapter 6, *Using the cell<sup>FRAP</sup> System*.

 To protect your specimen from photo-bleaching, close the laser shutter (by reversing step 5) whenever suitable, even if you interrupt observation only for a short time.

## 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. Adjustment necessary afterwards.	
Alignment of fiber at laser exit		

For details concerning the setup of the hardware, refer to your hardware manuals.

 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 FRAP 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; see the cell<sup>FRAP</sup> Hardware Manual.

### 5.2.2 Access to the Specimen without Deactivating the FRAP 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 FRAP 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.

### 5.2.3 Observing the Specimen Through the Ocular

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

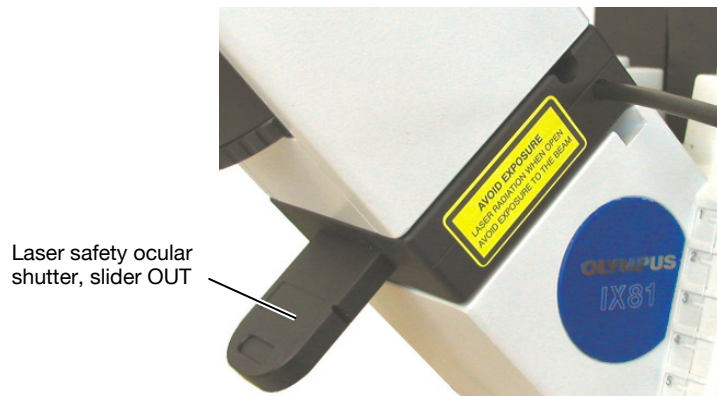
 Before observation via the ocular, make sure to have an adequate filter cube that quantitatively blocks the laser light inserted into the light path! The use of an inadequate or damaged dichroic mirror or emission filter can lead to intense, hazardous laser light exiting the ocular. This laser light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

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

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

---

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



- 
3. Hold a sheet of paper in front of the eyepieces.
- 



4. Open the laser safety shutters of the 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!



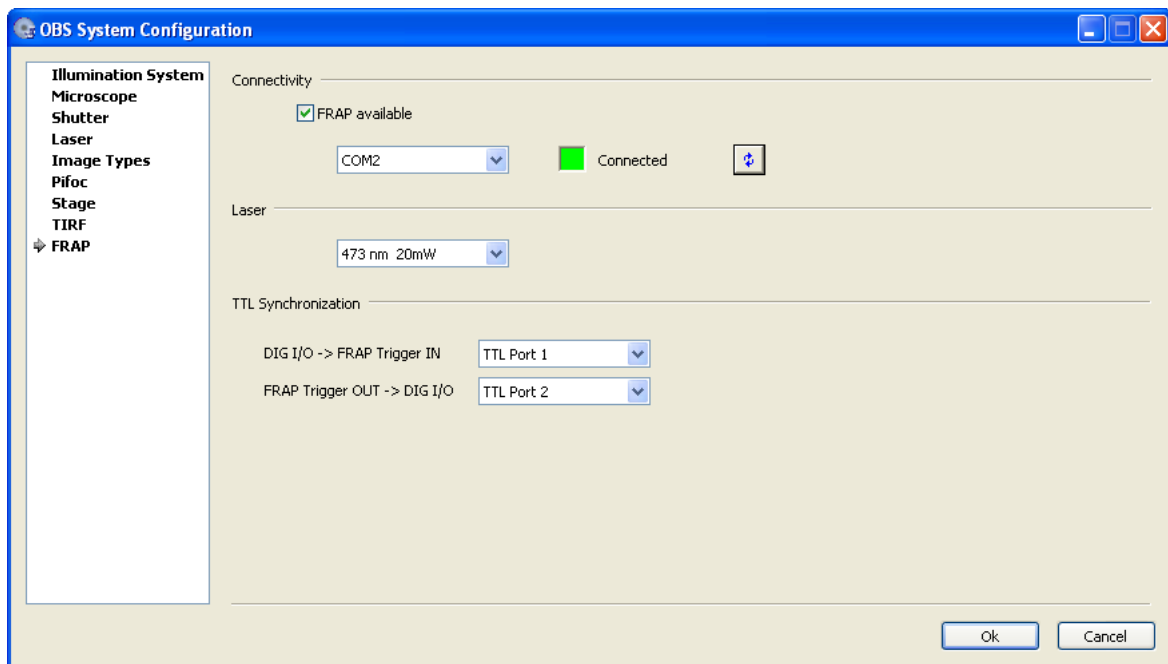
## 6 Operating the cell<sup>frap</sup> System

The cell<sup>frap</sup> scanning FRAP system and the Olympus Soft Imaging Solutions laser systems are fully integrated in the *xcellence* imaging software. This chapter describes the configuration and usage of the scanning FRAP system.

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## 6.1 System Configuration

1. Start the ObsConfig software to configure the excitation filters
- 
2. Make sure that the FRAP lasers are configured on pages **Microscope ▶ Laser** as described in the laser manual.
- 
3. Go to page **Microscope ▶ FRAP**.  
In case this page is not available right-click on OBS System Configuration (top left), open the **Components** context menu and activate **FRAP**.

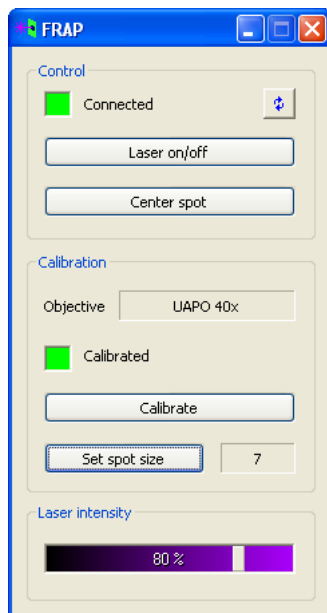


4. Click **FRAP available** to activate the **cell<sup>frap</sup>** unit.
- 
5. Select the PC serial port (COM port) connected with the **cell<sup>frap</sup>** control unit. Once selected correctly (and with the **cell<sup>frap</sup>** control unit powered up) the **Connected** field turns green.
- 
6. Select the **Laser** from the shortlist. Mind that this laser will not be eligible as light source for the **Image Types**.
- 
7. **TTL Synchronization, DIG I/O -> FRAP Trigger IN**. Select from the short list the TTL OUT connection at the **xcellence** imaging PC front panel that is connected with the **TRIGGER IN** of the **cell<sup>frap</sup>** control unit.

8. **TTL Synchronization, FRAP Trigger OUT -> DIG I/O.** Select from the short list the TTL IN connection at the xcellence imaging PC front panel that is connected with the **TRIGGER OUT** of the cell<sup>frap</sup> control unit.
- 
9. Exit the software with **OK**.

## 6.2 The FRAP GUI in xcellence

This interface is mainly meant for calibration purpose and for opening and closing the FRAP laser shutter. The use of cell<sup>frap</sup> in the context of "true" FRAP experiments is explained in the next Chapter 6.3, *cell<sup>frap</sup> in the Experiment Manager*.



### Control

**Disconnected / Connected.** The box turns green and reads **Connected** if the software detects the presence of the cell<sup>frap</sup> controller via the serial connection. The status can be update by clicking the **Update** button on the right side.

**Laser on/off.** Use this toggle button to turn the laser connected to the cell<sup>frap</sup> (and configured accordingly in OBS System Configuration) on or off (respectively, open or close its shutter).

**Center spot.** Click this button to move the scanner mirrors to their central position. This will set the laser spot to the central image position if the system is aligned correctly.

**Objective.** This field displays the currently active objective. The calibration needs to be performed for each objective that is to be used for the FRAP experiments. The currently used objective is listed here.

**Calibrated.** The status box turns green once the system is calibrated.

**i** The calibration is valid only for the objective in place during the calibration. A later manual change of the objective does not cause a change of the status display. However, a recalibration is required.

**Set spot size.** Click here to open the **Enter spotsize** window. Enter the spot **Diameter in pixel** and confirm with **OK**. The default value is set in dependence of objective magnification and NA as well as wavelength and pixel size. It presumes a perfectly focused laser. If the laser is defocused – which may well be done on purpose – the spot needs to be imaged and measured to determine its actual size.

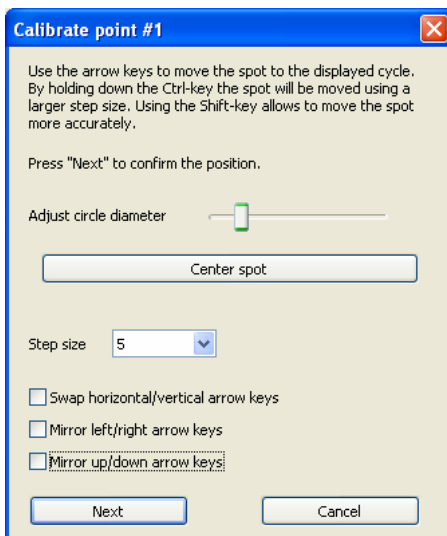


**Laser intensity.** Use the slider to attenuate the laser intensity.

**Calibrate.** Click here to start the calibration routine. The camera starts **Live** image acquisition, starts laser emission and the **Calibrate point #1** window opens.

The calibration procedure consists of two parts. First, the scanner positions corresponding to the four corners of the camera image are determined. Afterwards, in the so-called **Fine calibration**, a total of 16 scanner positions distributed over the camera field of view are referenced.

**i** The calibration is always done without binning on a full frame.



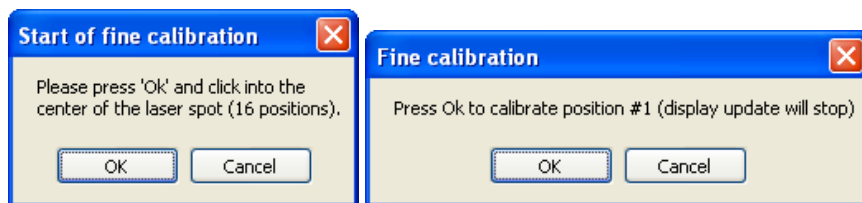
You may **Adjust circle diameter** by using the slide so that the red circle matches the laser spot in size. Mind that the laser spot size in the image not just depends on the laser focus but also on the **Brightness adjustment** settings in the **Camera control** window; see Chapter 4.2, *Camera Control*, in the **xcellence** software manual. It is often useful to set the **Max.** intensity clipping to 0 % here.

**Calibrate point #1.** In the top left corner of the Live image a red circle is marked. Use the arrow (cursor) keys to move the laser spot to this red circle. It is not required to do this with utmost precision

because the software will detect the bright spot when it is reasonably close. Increase the step size by keeping the Ctrl key pressed while using the arrow key. Keep the Shift key pressed to reduce it.

Once the spot is at the position of the red circle click **Next** to terminate the first calibration step. The red circle will now appear at the top right corner of the Live image and the **Calibrate point #2** window opens. Repeat the above procedure here and subsequently for **point #3** and **point #4** in the bottom left and right corners, respectively.

Once the fourth corner has been determined the **Start of fine calibration** window opens upon clicking **Next**. In this second part of the calibration routine the laser spot will move to 16 positions and needs to be moved on with the mouse cursor on and confirmed.



Click **OK**. The **Fine calibration** window opens and the laser spot will be positioned at the top left corner of the Live image. Move the tip of the mouse cursor over the laser spot and press the **Enter** key to confirm.

The laser spot will move to the second position at top half-left. Again, move the tip of the mouse cursor over the laser spot and press the **Enter** key to confirm.

Once all 16 positions have been confirmed the **Calibration** is finished. The calibration for the objective in use is stored and does not have to be repeated for this objective unless the camera is rotated relative to the C-mount.

## 6.3 cell<sup>frap</sup> in the Experiment Manager

### 6.3.1 FRAP Experiments



FRAP command icon

#### Types of experiments

**Advanced.** This selection gives access to all variable parameters.

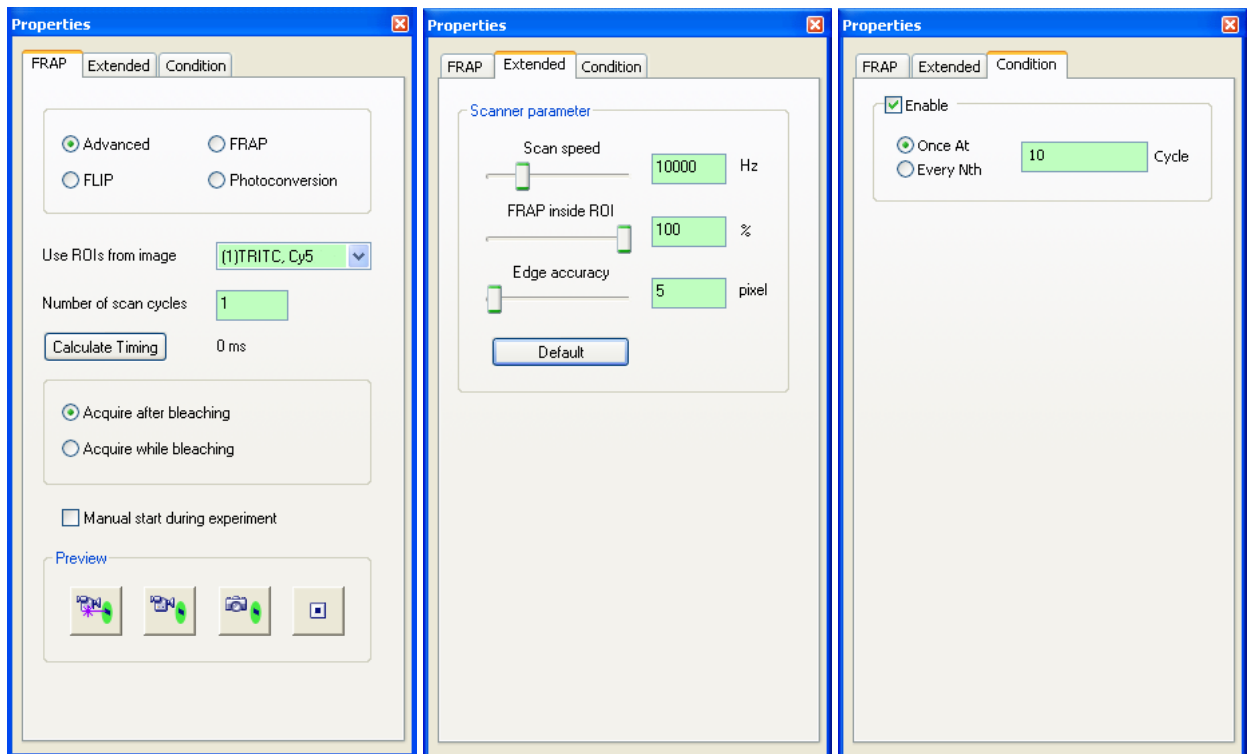
In order to simplify the interface partly preconfigured experiments can be selected. Here different sets of parameters are deactivated.

**FRAP (Fluorescence Recovery After Photobleaching).** This is the basic experiment where one or more ROIs are bleached once. Bleaching is always done **Once at Cycle 10** and the acquisition always done

**after bleaching.** The **Acquire** radio buttons and the **Condition** parameters are deactivated. **Manual start during experiment** is not possible.

**FLIP (Fluorescence Loss In Photobleaching).** In this experiment the ROIs are bleached in **Every Cycle** and acquisition always done **after bleaching**. The **Acquire** radio buttons and the **Condition** parameters are deactivated. **Manual start during experiment** is not possible.

**Photoconversion.** This experiment is identical to FRAP with the exception that the acquisition always done **while bleaching**. The **Acquire** radio buttons and the **Condition** parameters are deactivated. **Manual start during experiment** is not possible.



**Use ROIs from image.** Select the image containing the ROIs to be bleached from the shortlist. It lists only images that contain ROIs.

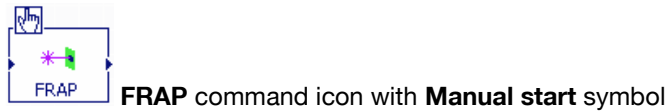
**Number of scan cycles.** Set here the number of times the ROIs are to be scanned in each **(Time Loop) Cycle**. The default is 1 but one may decide to scan several times, for example with reduced laser intensity. By doing so the influence of timing differences between the bleaching of the first positions of a ROI and the last positions is reduced.

**Calculate Timing.** Click here so that the software calculates how long the scan will take. The value is displayed next to the button. By changing the **Number of scan cycles** you may thus determine how long the bleaching will last.

**Acquire after photobleaching.** With this option the following image acquisition is synchronized with the end of the ROI scan(s). The laser spot will not be visible in this image. This option is automatically selected in case of **FRAP** and **FLIP** experiments.

**Acquire while photobleaching.** Here image acquisition and ROI scan start simultaneously. The trace of the scanning laser spot will be visible in the image. This option is automatically selected in case of **Photoconversion** experiments.

**Manual start during experiment.** This check box is active only if the **Advanced** experiment is selected. If the option is checked the command icon shows a hand symbol. The command icon turns into a button.



### What will happen?

After the start of the experiment the FRAP command icon will simply be ignored until clicked by the user. Clicking is possible at any time and will immediately start the ROI scan independently of the position of the command icon in the experiment plan and independently of the current advance of the experiment.

### Preview



Click here to start a live preview simultaneously with a laser scanning cycle. The scanning laser spot will be visible in the beginning.



Click here to start a laser scanning cycle with a live preview following immediately after the scan is finished.



Click here to start a laser scanning cycle with a single snapshot following immediately after the scan is finished.



Click here to stop the **Live view**.

### Extended

**Scan speed.** This determines the duration of a scan and thus the time that the laser rests at each position during the scan. Set the scan speed by use of the slider or by typing a value. The speed is expressed as frequency in Hz. The default value is 10 kHz (10.000 Hz).

**FRAP inside ROI.** Mind that the ROIs drawn in the image will be "filled" with positions of the laser spot and thus the form of the ROIs will only be approximated. This parameter defines how much the outer laser spot positions may overlap with the ROI borders. Set the percentage by use of the slider or by typing a value. The default value is 100%.

**Edge accuracy.** The FRAP scanner controller composes the ROIs to be scanned out of sub-rectangles. The smaller the sub-rectangles are the higher is the resulting correspondence with the ROIs. Set the accuracy by use of the slider or by typing a value. The default value is 5 pixels, the range 2 pixels.

**Default.** Click here to reset the defaults.

### Condition

These options are active only if the **Advanced** experiment is chosen.

**Enable.** Check here to use the options. Otherwise the command is executed in each **Time loop** cycle.

**Once at ... Cycle.** This option is used in typical FRAP experiments. Set the cycle number when the FRAP scan is to be executed.

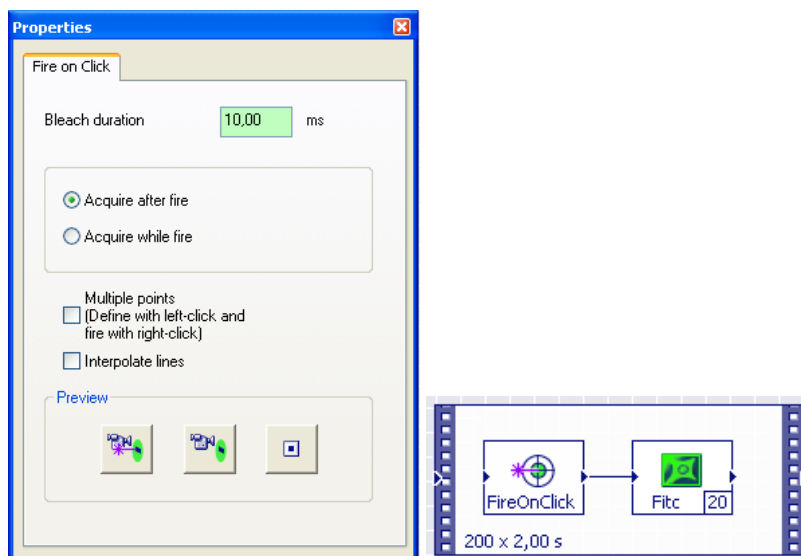
**Every Nth ... Cycle.** This option is used in typical FLIP experiments. Set the interval between the bleaching scan if **time loop** cycles with and without scan shall alternate.

## 6.3.2 Fire On Click



**Fire On Click** command icon

This command icon allows applying a laser flash at will during the experiment and at any spot within the image. It can be repeated as often as wanted. This allows carrying out bleaching or photoactivation experiments where it is not known before hand when and where an object to be exposed to the laser will appear.



The system will **Fire** after a **Click** the next time the **Fire On Click** command is being cycled through in the experiment plan. Let's take the following Experiment plan; see below. The **Fire On Click** command

is the first one within each cycle. At one point in time during a certain cycle a **Click** is being applied. The Fire will only be carried out as soon as the next cycle is being started. Thus, while the manual **Click** is unsynchronized, of course, the **Fire** initialized by the FRAP controller is not.

The software "burns" a marker into the image following a "Fire" each time.

**Bleach duration.** Set here the duration of the laser flash.

**Acquire after fire.** With this option the following image acquisition is synchronized with the end of the ROI scan(s). The laser spot will not be visible in this image.

**Acquire while fire.** Here image acquisition and ROI scan start simultaneously. The trace of the scanning laser spot will be visible in the image.

**Multiple points.** This option allows to select several spots in the image with left mouse clicks– the software internally generates a list of XY positions – and flash them all subsequently as fast as possible upon a right click.

**Interpolate lines.** This option is available only if option **Multiple points** is checked. Activate this option to do "line bleaching" instead of bleaching individual spots. Apply a series of (left) **Clicks** on certain points of a structure. The display will show a polygon line instead of spots. Start the **Fire** with a right (!) mouse click. The scanner will move the laser beam along the line – and not switch it off between the spots as in the usual mode.

**Preview: Live view while bleaching, Live view after bleaching, Stop FRAP preview.** See previous chapter for details.

### 6.3.3 Pattern Bleaching – Fluorescence Speckle Microscopy



**Pattern Bleaching** command icon

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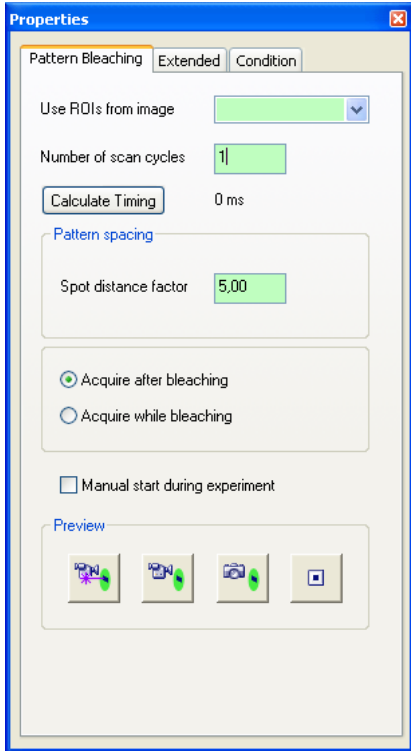
In this experiment the laser spot positions are set with predefined gaps in between. The resulting pattern of bleached or photoactivated spot makes movements of structures relative to each other quite visible.

Most parameters and options are explained in detail in Chapter 6.3.1, *FRAP Experiments*.

#### **Pattern spacing**

**Spot distance factor.** Set the value here. The **Spot size** is being multiplied with the **Spot distance factor** in order to define a certain distance between each spot of the pattern.

Remember that the **Spot size** is defined via **Set spot size** in the **FRAP** window with the default value presuming a perfectly focused laser; see Chapter 6.2, *The FRAP GUI in xcellence*. The pattern is most regular if rectangular ROIs are being used. Other types of ROIs need to be composed of sub-rectangle as described for the command **Edge accuracy** in Chapter 6.3.1, *FRAP Experiments*. Here the pattern of spots will be more irregular.



# 7 Appendix

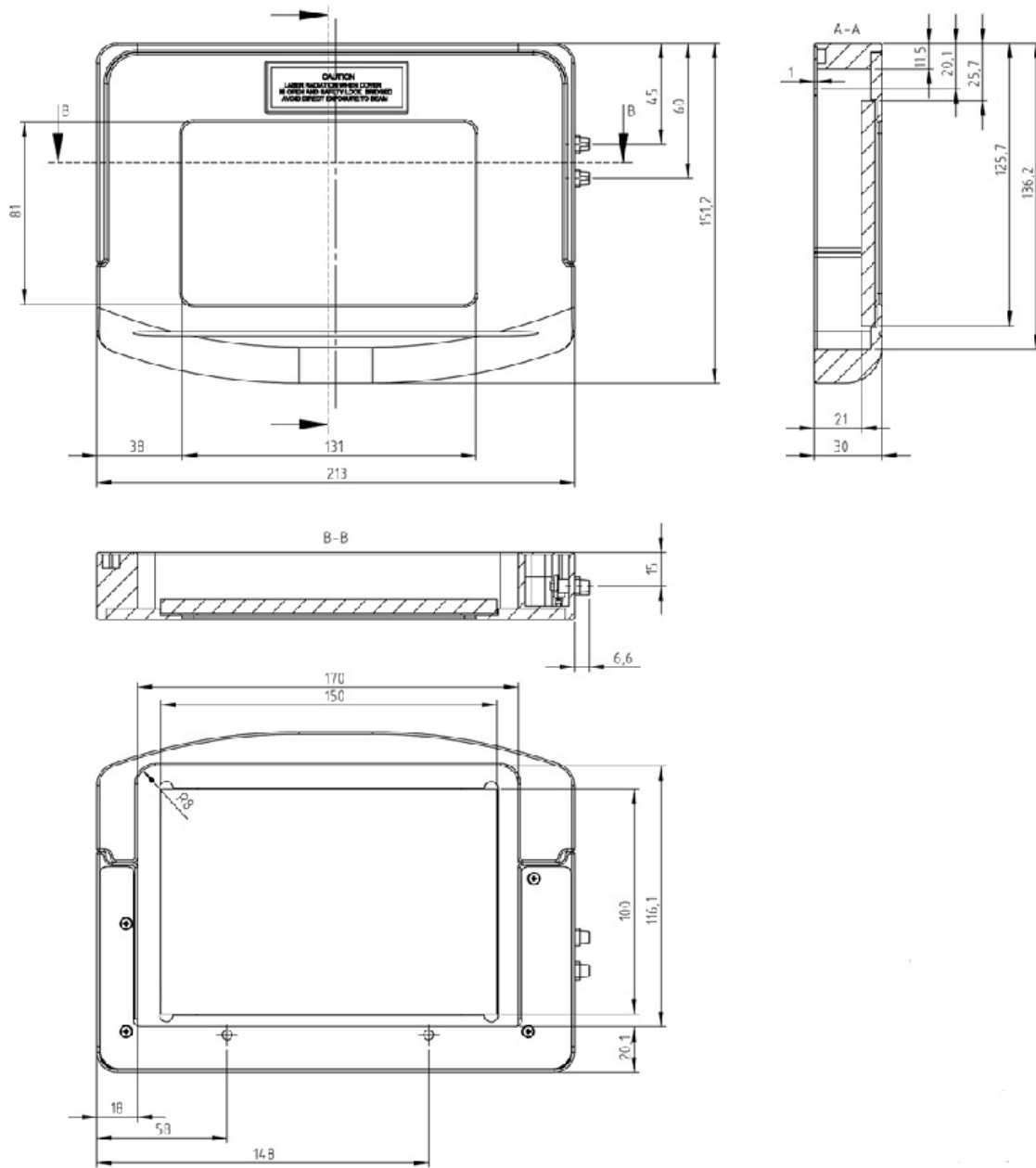
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This chapter lists specifications and technical data of the cell<sup>frap</sup> system.

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## 7.1 Laser Safety Stage Cover Dimensions

The laser safety stage cover must be secured to the microscope stage with the M4x5 screws provided. If the microscope stage does not have the necessary pre-drilled holes, this simple modification must be performed before using the system. The following drawings show all dimensions necessary for such a modification.









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