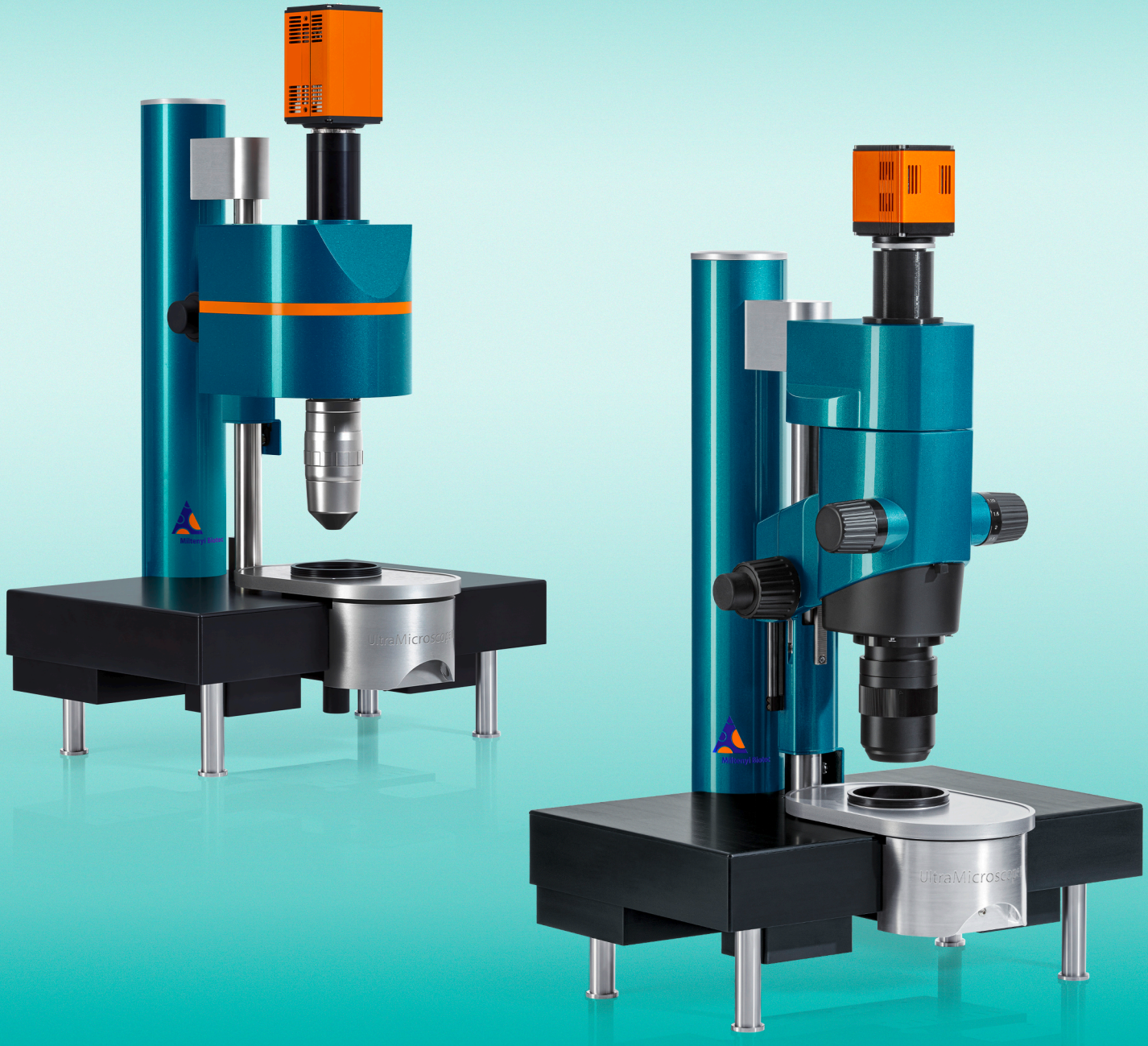
Miltenyi Biotec

UltraMicroscope II Imaging System

User manual



UltraMicroscope II Imaging System

User manual

Original instructions

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Read the chapter **Important safety information** and all other information in this user manual before using the instrument

⚠ WARNING

Before using the UltraMicroscope II Imaging System, read the chapter **Important safety information** and all other information contained in this user manual, including any safety and operating instructions. Pay special attention to all warnings displayed on the instrument. Failure to read and follow these guidelines could lead to improper or incorrect usage and result in damage to the instrument. Improper usage could also cause severe personal injury, death, unpredictable results, instrument malfunction, and premature wear to components shortening the lifetime of the instrument. Such actions may void your warranty. Keep the user manual and any other safety and operating instructions provided with the instrument in a safe place accessible to all users for future reference.

If you have a serious concern regarding the safe use of your instrument, contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Technical Support.

Content

1 Important safety information

General safety instructions

This chapter provides important information for your personal safety and the correct use of the instrument. Read and observe all instructions carefully before proceeding with the installation and use of the instrument. Observe general safety practices in addition to this user manual.

This instrument is a Class 3B laser device that requires special safety measures.

- Use this instrument only as indicated in this user manual to avoid personal injury and property damage.
- Keep this user manual in a place that is always accessible to all users.
- Observe local working area safety instructions and laboratory policies, as well as standards for health, safety, and prevention of accidents.
- Ensure safe working conditions and provide sufficient lighting.
- Safe working is only possible if solely trained personnel use the instrument. The training is a permanent duty of the operator.
- Ensure that the power switch and the plug of the power cable of the instrument are easily accessible.
- To entirely disconnect the instrument from the power supply, unplug the power cable.
- Ensure that the power cable plug is close to the user.
- In case of severe accidents, damages to the instrument, or if smoke or flames appear, cut the power supply immediately.
- If it is necessary to cut the power supply, unplug the cable from the power outlet and contact an authorized Miltenyi Biotec service provider or Miltenyi Biotec Technical Support.
- Check the instrument before each use for visible damage.
- Do not use the instrument if it has been dropped or is damaged. Contact Miltenyi Biotec Technical Support immediately.

Hazard levels

Signal words are used to warn against hazardous situations and property damages. The following signal words are used in this user manual.

⚠ WARNING or WARNING! indicates a hazardous situation that, if not avoided, could result in death or serious injury.

⚠ CAUTION or CAUTION! indicates a hazardous situation that, if not avoided, could result in minor or moderate injury. It is also used to warn against unsafe practices.

NOTICE or NOTICE indicates information considered important but not hazard related (e.g. messages relating to property damage).

Symbols


SAFETY WARNING: THE DOCUMENTATION MUST BE CONSULTED IN ALL CASES WHERE THIS SAFETY SYMBOL IS USED ON THE INSTRUMENT


LASER RADIATION HAZARD


WEEE (WASTE OF ELECTRICAL AND ELECTRONIC EQUIPMENT)


READ THE USER MANUAL BEFORE USING THE INSTRUMENT

ON (POWER ON)


OFF (POWER OFF)


WEIGHT

ORDER NUMBER

SERIAL NUMBER

TYPE NUMBER


MANUFACTURER


DATE OF MANUFACTURE


EUROPEAN CONFORMITY MARKING

Safety labels

Observe the hazard areas and safety symbols of the instrument.

- Keep safety labels and safety markings clean and legible.
- Inspect the safety labels and safety markings regularly and replace them if they are not legible or identifiable from a safe viewing distance.
- Contact Miltenyi Biotec for replacement labels.

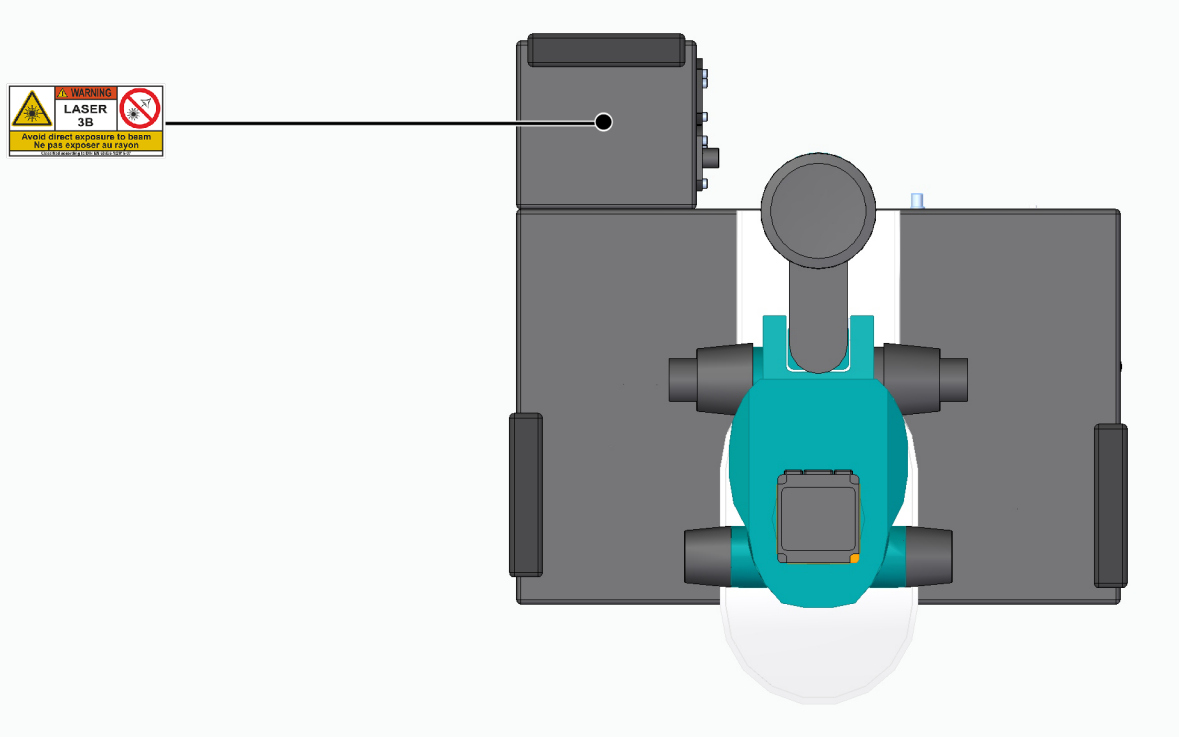


Figure 1.1: Hazard areas and safety symbols on the top side of the instrument

Electrical hazards

⚠ WARNING

Electric shock, short circuit, overheating, fire, and explosion could result in death or serious injury.

Protective housing

The housing of the instrument reduces the risk of an electric shock and short circuits.

- Do not disassemble the instrument.
- Do not use the instrument if it has been dropped or is damaged.

Cables and power supplies

The instrument has a three-wire electrical grounding plug with a third pin for grounding. Using the instrument with other than the supplied cables is potentially hazardous.

- Only use the included power cable.
- Do not use the instrument if the power cable is damaged.
- Only insert the plug into a grounded electrical outlet. Do not try to insert the plug into a non-grounded electrical outlet.
- Only use the power supply provided. If you have questions about the type of power source to use, contact Miltenyi Biotec Technical Support or your local electricity supplier.

Liquids inside the instrument

Liquids inside the instrument can cause short circuits.

- Do not use the instrument if liquids have entered the inside of the instrument.
- Protect the instrument against spilled liquids.
- Do not allow liquids to enter the inside of the instrument.
- Clean up spilled liquids immediately.
- Unplug the instrument before cleaning it.
- Use only small amounts of cleaning agents on a soft cloth to wipe the instrument. Do not spray or pour liquid cleaning agents onto or into the instrument.

Humid and dusty environments

A change in the environmental temperature may cause condensation inside the instrument. Humidity, condensation, or dust inside the instrument can cause short circuits.

- Use the instrument only indoors.
- Do not use the instrument in a wet location or areas with high humidity or condensation.
- After moving the instrument from a cold environment to room temperature, wait for the instrument to dehumidify before using it.

Biological hazards

⚠ WARNING

Contamination or infection could result in death or serious injury depending on the material used.

Biological material

All biological material must be considered potentially infectious.

- Wear personal protective equipment (such as gloves, safety glasses, etc.) as indicated in the safety data sheet for the particular substance.
- If hazardous or potentially infectious material has been spilled or leaked from the system, decontaminate the affected area.
- Operate the instrument in a biological safety cabinet suitable for the used specimen if hazardous or potentially infectious materials are processed.
- Take care when handling samples and reagents.

Waste

All liquid and solid waste must be considered hazardous.

- Observe local regulations regarding waste disposal.

Equipment damage

Defective or inadequate equipment can cause a biological hazard. Some decontamination methods could damage the instrument.

- If parts of the instrument are damaged, unplug and do not use the instrument until damaged parts are replaced. Contact Miltenyi Biotec Technical Support, if necessary.
- Observe the instructions in the data sheet for each consumable.
- Use only 70% ethanol or isopropyl alcohol for disinfection.

Chemical hazards

⚠ CAUTION

Substances and reagents can be hazardous.

Benzyl ether could irritate the respiratory tract.

- All safety measures in section **Biological hazards** also apply to any hazardous substances and reagents that may be present in the sample.
- Use any substances and reagents only as stated in the respective safety data sheet.
- Operate the instrument in a fume hood or with a fume extraction arm if hazardous substances and reagents are processed.
- Avoid storing cuvettes filled with imaging solution because of the evaporation of volatile chemicals and resulting changes. Water attraction of chemicals and resulting change of the pH value is possible.

Mechanical hazards

⚠ CAUTION

Risk of crushing or cutting.

Heavy instrument

The instrument could fall down and cause serious injury to hand or foot.

- Only use tables that support a weight of 250 kg.
- Do not install the instrument yourself.
- Only trained Miltenyi Biotec personnel or personnel authorized by Miltenyi Biotec are allowed to install the instrument.

Radiation hazards

⚠ WARNING

Direct exposure to laser beam could result in eye or even skin injury.

The instrument is equipped with a Class 3B laser product (Laser BC) or Class 4 laser product (supercontinuum WLL) according to standard IEC 60825-1:2014.

- Do not remove the protective housing.
- Do not stare into laser beam.
- Do not insert reflective objects into the cuvette.
- Wear laser safety goggles.
- Switch off immediately if there is any indication of laser radiation outside the instrument.

Ergonomic hazards

⚠ CAUTION

Risk of tearing or straining muscles.

- Do not move the instrument yourself.
- Only trained Miltenyi Biotec personnel or personnel authorized by Miltenyi Biotec are allowed to transport the instrument.

Environmental hazards

⚠ CAUTION

Tripping could result in injuries.

- Check the environment around the instrument before each use for tripping hazards, such as cables lying on the floor.

⚠ CAUTION

Slippery surfaces could result in injuries.

- Ensure that the floor around the instrument is clean.

Servicing and transportation

Servicing

Improper servicing or repair of the instrument or use of unauthorized parts can cause malfunction of or damage to the instrument. This can cause hazards to users.

- Unless otherwise specifically stated in this user manual or other documentation by Miltenyi Biotec, do not service the instrument yourself.
- Servicing and repair must be performed by Miltenyi Biotec certified and qualified service personnel.
- If the instrument needs servicing, decontaminate the instrument to remove any hazardous material.
- If you have questions regarding proper decontamination or shipment, contact Miltenyi Biotec Technical Support for assistance.
- Only use accessories and upgrades recommended by Miltenyi Biotec.
- Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and service contracts, or refer to **www.miltenyibiotec.com/support**.

Transportation

The instrument must be transported with care in packaging specified by Miltenyi Biotec. Internal damage could occur if the instrument is subjected to excessive vibration or if it is dropped. If the instrument needs to be shipped back to the manufacturer for service, contact Miltenyi Biotec for instructions and packaging materials.

Disposal

WASTE OF ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) CUSTOMER INFORMATION

Dispose of your end-of-life Miltenyi Biotec products in accordance with the applicable WEEE and hazardous waste disposal legislation, which may differ by country or region. Electrical equipment may contain hazardous substances that may have a serious detrimental effect on the environment and/or human health. All equipment must be specifically collected and treated by designated waste facilities and by qualified WEEE compliance schemes. By ensuring that you dispose of your unwanted electrical and electronic equipment according to the applicable WEEE and hazardous waste disposal legislation, you are helping to preserve our natural resources and protect human health. Miltenyi Biotec is committed to protecting the environment. Miltenyi Biotec offers product end-of-life return programs in many countries and partners with licensed WEEE compliance schemes throughout the world. Miltenyi Biotec takes back your end-of-life Miltenyi Biotec equipment for recycling free of charge. The terms and availability of this offer vary by geography because of differences in regulatory requirements. Note that, depending on the type and use of your equipment, additional requirements may apply. Before shipping the instrument back to the manufacturer for disposal, decontaminate the instrument to remove any hazardous material. For more information or if you wish to dispose of your end-of-life Miltenyi Biotec equipment, contact your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support.

2 Introduction

2.1 The UltraMicroscope II Imaging System

Understanding spatially complex biological systems, like the vasculature of an entire organ, requires the availability of high-resolution information on their tissue architecture. The light sheet microscopy can provide this information.

To visualize larger samples with the same z-resolution, the UltraMicroscope II Imaging System uses dynamic horizontal focusing. Samples remain stationary while the focus position moves, shifting the narrow range of detection horizontally to generate a series of snapshots. These snapshots are blended into one high-quality image by the software.

The instrument uses two opposing sets of three light sheets, each positioned at a slightly different angle. All six light sheets converge on the focal plane to illuminate all areas of the sample and minimize shadow artifacts. Improved optional sectioning gets the most out of the sample.

The UltraMicroscope II Imaging System can be equipped with one of the following modules:

- the Zoom Body Module for easy handling in a multi-user environment
- the Super Plan Module that can deliver unprecedented image resolution and image quality, and an automated magnification changer

The Super Plan Module can be mounted directly to the focusing unit of existing systems.

The UltraMicroscope II Imaging System can be upgraded with an *in vivo* sample cuvette, which allows setting and maintaining of a constant immersion medium temperature and CO_2/O_2 atmosphere within the cuvette.

2.2 Intended use

The UltraMicroscope II Imaging System is a semiautomated light sheet microscope for imaging large or multiple samples. The sample must be examined in the supplied cuvette. Unauthorized modifications of the cuvette or the instrument as a whole are not permitted for safety reasons. The cuvette must only be filled with MACS Imaging Solution or another imaging solution approved by Miltenyi Biotec. The instructions for operation, maintenance, and service described in this user manual must be observed.

The UltraMicroscope II Imaging System is for research use only in a laboratory environment. Only laboratory professional users are allowed to use the instrument.

2.3 Instrument description

The UltraMicroscope II Imaging System comes in two configurations:

- Zoom Body
- Super Plan

For the Zoom Body configuration, there are two laser configurations and two camera configurations available. There is only one objective and one dipping cap available.

For the Super Plan configuration, there are two laser configurations and two camera configurations available. There are three different objectives and four dipping caps available. This guarantees a higher image quality.

2.3.1 Front view Zoom Body configuration

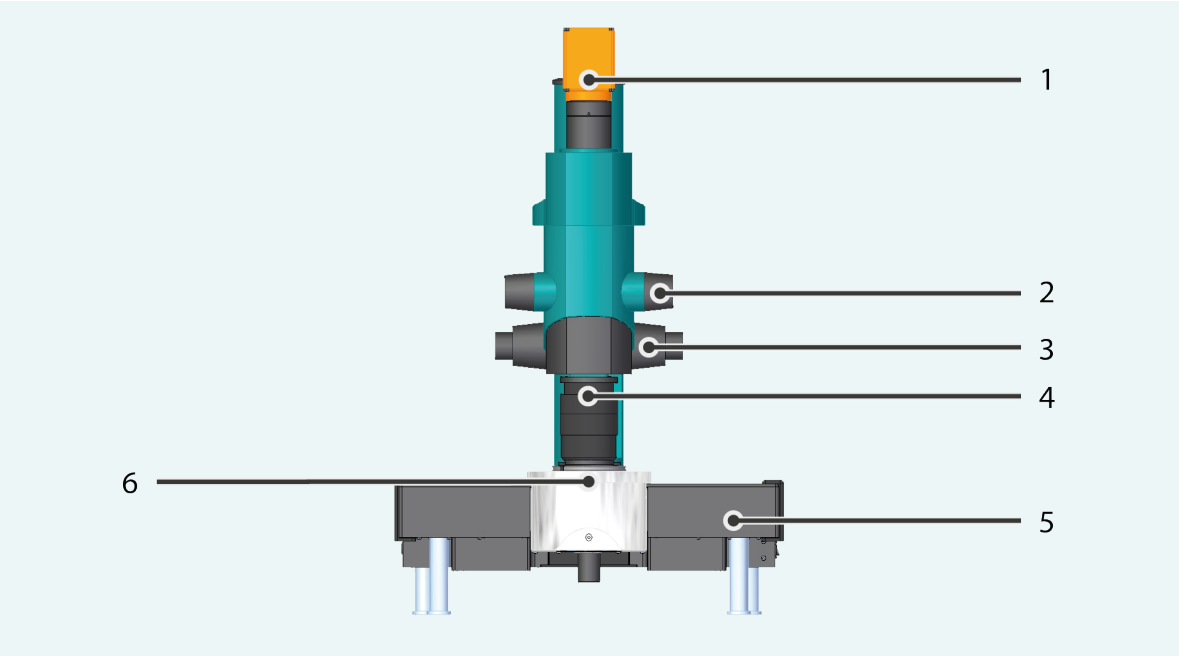


Figure 2.1: Front view of the UltraMicroscope II Imaging System Zoom Body configuration

1	camera	**4**	objective lens
2	zoom knob	**5**	light sheet forming unit
3	focusing unit	**6**	cover plate

2.3.2 Rear view Zoom Body configuration

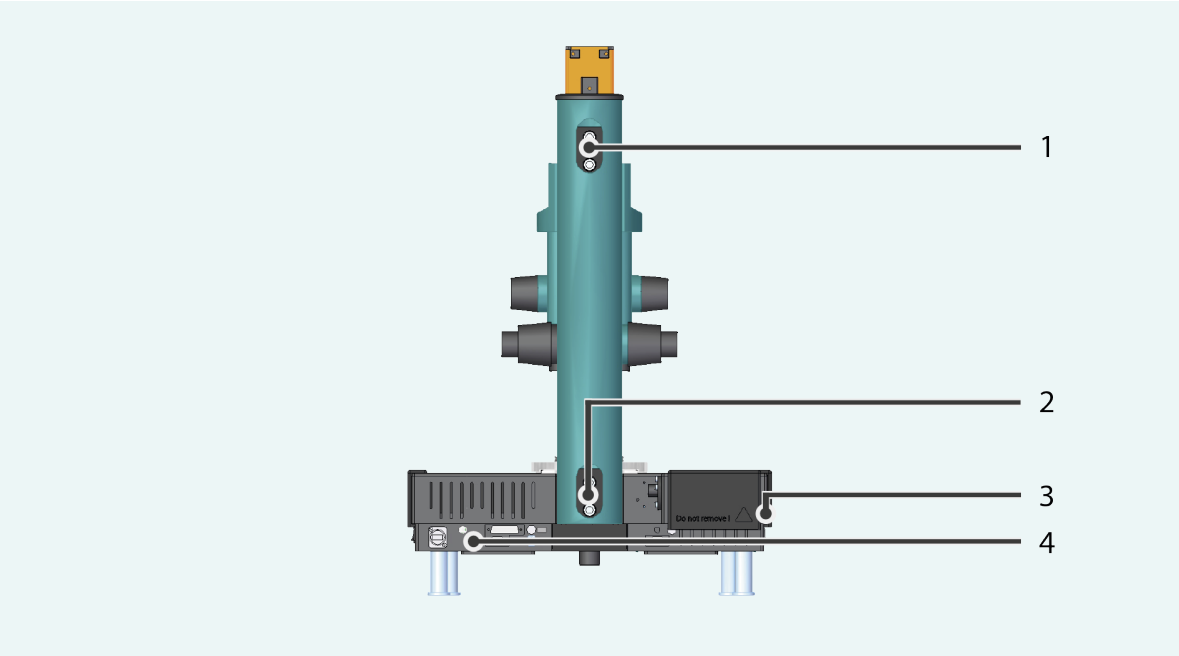


Figure 2.2: Rear view of the UltraMicroscope II Imaging System Zoom Body configuration

1	cable duct	**3**	type label
2	cable duct	**4**	electronic interface

2.3.3 Front view Super Plan configuration

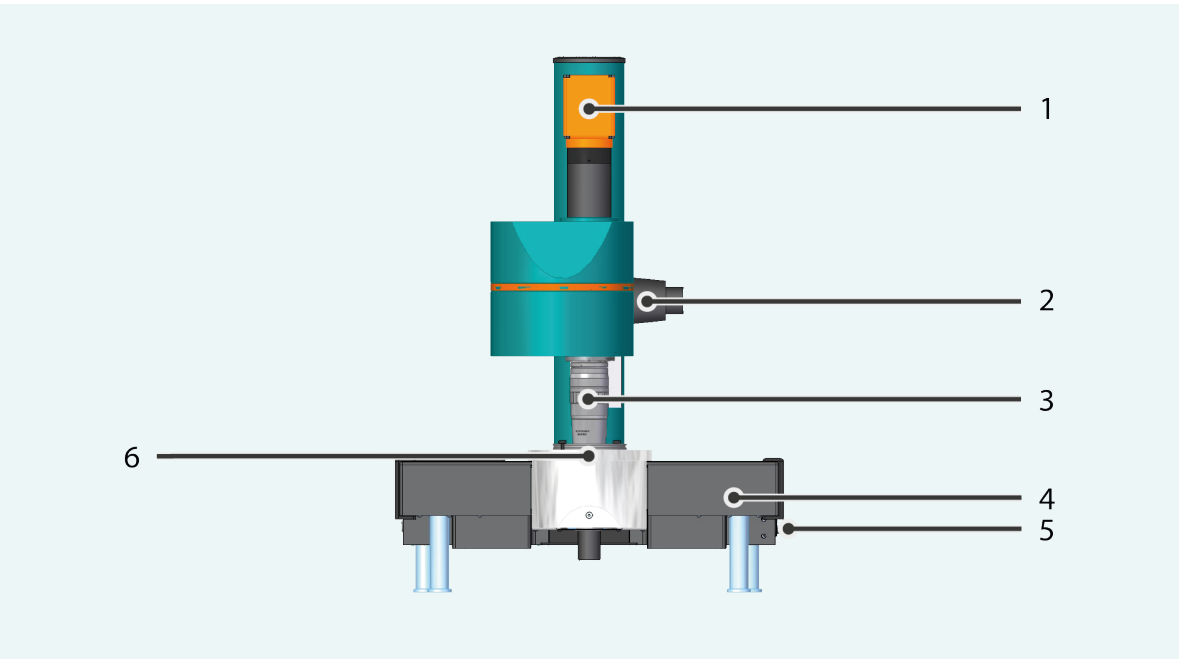


Figure 2.3: Front view of the UltraMicroscope II Imaging System Super Plan configuration with position numbers

1	camera	**4**	light sheet forming unit
2	focusing unit	**5**	power switch
3	objective lens	**6**	cover plate

2.3.4 Rear view Super Plan configuration

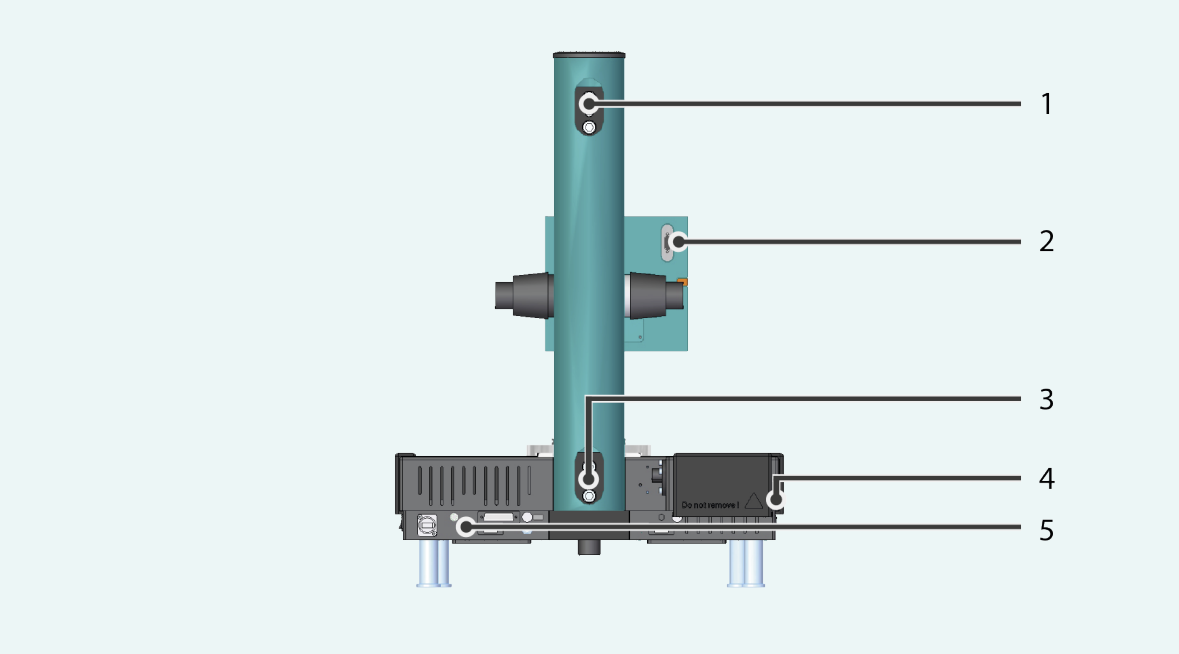


Figure 2.4: Rear view of the UltraMicroscope II Imaging System Super Plan configuration

1	cable duct	**4**	type label
2	controller connection	**5**	I/O ports
3	cable duct		

2.3.5 Detail view of electronic interface

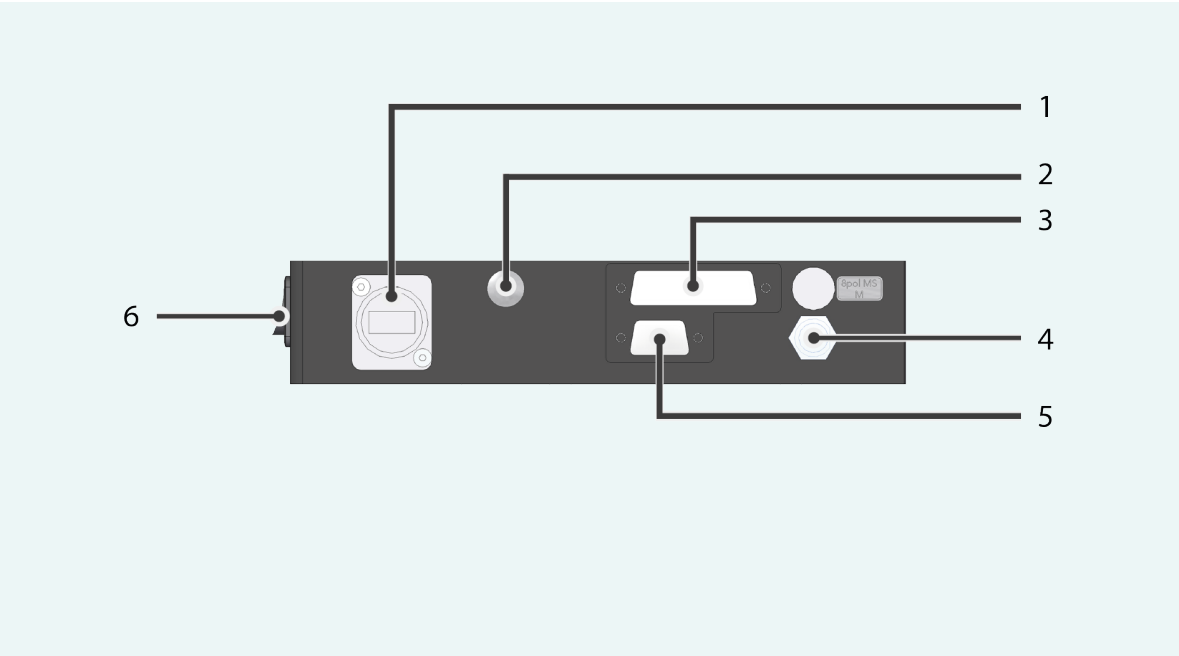


Figure 2.5: Detail view of the I/O ports and power switch

1	power supply	**4**	camera trigger
2	jog wheel	**5**	PC connection
3	detection unit	**6**	power switch

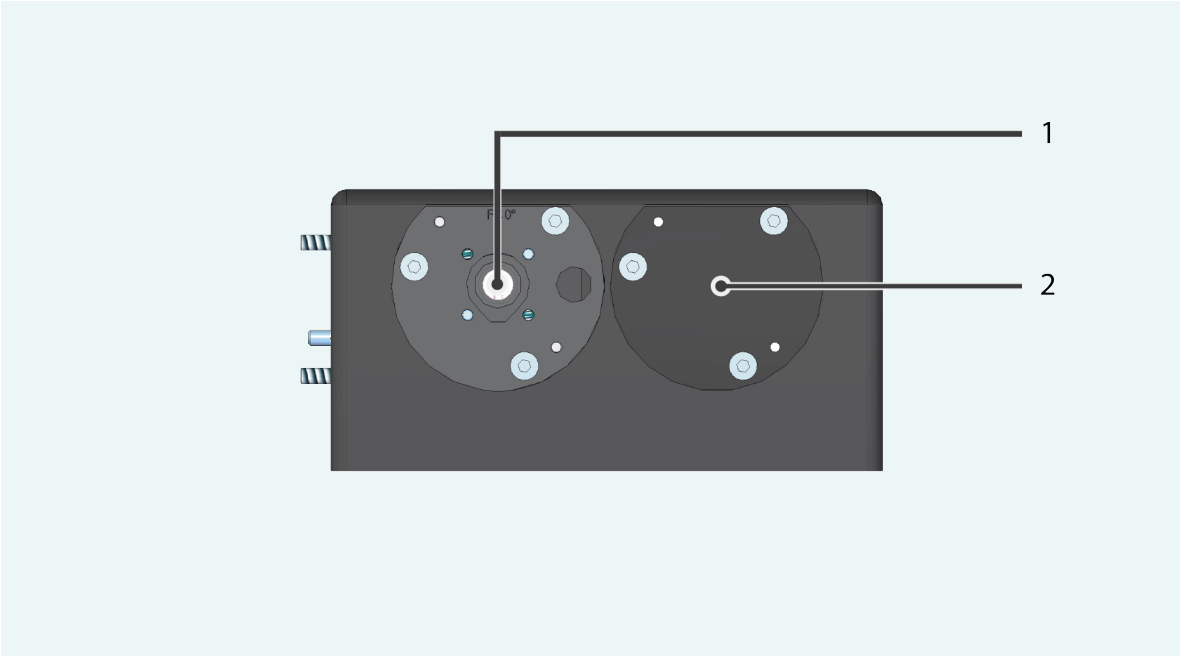


Figure 2.6: Detail view of the coupling box

1	laser fibre (1)	**2**	optional: laser fibre (2)

3 Installation

3.1 Components Zoom Body configuration

The Beam Combiner (BC) and the white light laser (WLL) configuration are delivered with lasers, objective lenses, and emission filters, as specified when ordering the instrument. Additional accessories and consumables developed specifically for the system are available separately. See **Table 3.2** and **Table 3.5**. For details, visit **www.miltenyibiotec.com** or contact your local sales representative.

Components included in the delivery		
	Beam Combiner (# 130-133-575)	**White light laser (# 130-133-570)**
light source	up to 5 lasers of choice*	supercontinuum white light laser
objective lens	2x	2x
emission filter (Ø 32 mm)	up to 7 filters of choice*	up to 7 filters of choice*
excitation filter (Ø 12.5 mm)	-	up to 8 filters of choice*
camera	4.2 Mpx sCMOS camera	4.2 Mpx sCMOS camera
cuvette	×	×
jog wheel	×	×
power supply for instrument	×	×
workstation PC with preinstalled software	×	×
keyboard and mouse	×	×
headset	×	×
HD webcam	×	×
monitor	×	×

Table 3.1: Components included in the delivery (* The delivery of these items depends on the ordered configuration.)

3.2 Accessories Zoom Body configuration

Product name	Order no.
Objective lens with dipping cap	
Objective lens Olympus MVPLAPO 2x	150-000-490
Dipping cap for Zoom Body configuration Objective 2x 0.5 NA WD 5.7 mm	130-133-638
Various accessories	
Alignment tool for UltraMicroscope II Imaging System	130-133-644
Sample holder set for UltraMicroscope II Imaging System	130-133-642
Cuvette for UltraMicroscope II Imaging System	130-133-635

Table 3.2: Optional accessories available for the instrument

3.3 Components Super Plan configuration

The Beam Combiner (BC) and the white light laser (WLL) configuration are delivered with lasers, objective lenses, and emission filters, as specified when ordering the instrument. Additional accessories and consumables developed specifically for the system are available separately. See **Table 3.4** and **Table 3.5**. For details, visit **www.miltenyibiotec.com** or contact your local sales representative.

Components included in the delivery		
	Beam Combiner (# 130-133-575)	**White light laser (# 130-133-570)**
light source	up to 5 lasers of choice*	supercontinuum white light laser
objective lens with dipping cap	up to 7 objective lenses*	up to 7 objective lenses*
emission filter (Ø 43 mm)	up to 7 filters of choice*	up to 7 filters of choice*
excitation filter (Ø 12.5 mm)	-	up to 8 filters of choice*
camera	4.2 Mpx sCMOS camera*	4.2 Mpx sCMOS camera*
cuvette	×	×
jog wheel	×	×
power supply for instrument	×	×
workstation PC with preinstalled software	×	×
keyboard and mouse	×	×
headset	×	×
HD webcam	×	×
monitor	×	×

Table 3.3: Components included in the delivery (* The delivery of these items depends on the ordered configuration.)

3.4 Accessories Super Plan configuration

Product name	Order no.
Objective lenses with dipping caps	
Objective lens 1.1x NA 0.1 MI PLAN with two dipping caps for organic/aqueous buffers	130-133-625
Objective lens 4x NA 0.35 MI PLAN with dipping cap for water	130-133-602
Objective lens 4x NA 0.35 MI PLAN with dipping cap for aqueous buffers	130-133-611
Objective lens 4x NA 0.35 MI PLAN with dipping cap for organic solvents	130-133-601
Objective lens 12x NA 0.53 MI PLAN with dipping cap for water	130-133-605
Objective lens 12x NA 0.53 MI PLAN with dipping cap for aqueous buffers	130-133-608
Objective lens 12x NA 0.53 MI PLAN with dipping cap for organic solvents	130-133-606
Dipping caps	
Dipping cap water for objective lens 4x MI PLAN	130-133-637
Dipping cap aqueous buffers for objective lens 4x MI PLAN	130-133-639
Dipping cap organic solvents for objective lens 4x MI PLAN	130-133-640
Dipping cap water for objective lens 12x MI PLAN	130-133-631
Dipping cap aqueous buffers for objective lens 12x MI PLAN	130-133-633
Dipping cap organic solvents for objective lens 12x MI PLAN	130-133-632
Cameras	
4.2 Mpx sCMOS camera	150-000-552
Various accessories	
Alignment tool for UltraMicroscope II Imaging System	130-133-644
Sample holder set for UltraMicroscope II Imaging System	130-133-642
Cuvette for UltraMicroscope II Imaging System	130-133-635

Table 3.4: Optional accessories available for the instrument

3.5 Consumables

Product name	Order no.
MACS Clearing Kit	130-126-719
MACS Imaging Solution	130-126-335

Table 3.5: Consumables that have to be ordered separately

3.6 Unpacking and installation

Protect the instrument from shock and vibration. Do not place vibrating devices, such as vortex mixers, on the same table as the instrument. This could impair the quality of the results.

Only Miltenyi Biotec service personnel are allowed to unpack and install the instrument. Contact Miltenyi Biotec Technical Support for further information.

Before installation, ensure that your site is properly prepared. The instrument must be installed on an optical table with vibration isolation that can carry a weight of at least 250 kg. Allow a minimum of 20 cm free space to the rear side and to both sides of the instrument. Allow a minimum of 100 cm free space in front of the instrument. All sides of the instrument must be accessible for service and maintenance. Due to vibrations, an adjacent desk is recommended for the laser source, the workstation PC and its components.

4 Operation

4.1 Switching on the instrument

1. Ensure that all components are connected to the power supply.
2. Switch on the external laser module. The key switch is located on the laser module, refer to manufacturer documentation.
3. Switch on the instrument. The power switch is located on the right-hand side of the instrument, refer to **Instrument description on page 14**.
4. Switch on the camera.
5. Switch on the workstation PC and sign in on the Windows system with your user credentials.
6. Open the ImSpector software by double-clicking the program icon. The switched on devices are registered and checked.

4.2 Switching off the instrument

1. Close the ImSpector software.
2. Shut down the Windows system.
3. Switch off the camera.
4. Switch off the instrument.
5. Switch off the external laser module.

4.3 Graphical user interface

	Name	Function	Location
	**Save Settings**	saves all settings	toolbar
	**Start Measurement**	starts the defined measurement	Measurement Wizard
	**Start Live Preview**	starts a live preview	Measurement Wizard
	**Autosave**	activates autosave for the selected device	Measurement Wizard
	**Adjust window/level**	adjusts the contrast of the colormap automatically to the minimum and maximum pixel value within the whole image or a shown region of interests	stack window

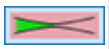
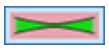
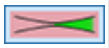
	Name	Function	Location
	**ROI for active profile**	shows or hides the region of interest (ROI)	stack window
	**Rayleigh ROI**	switches on the light sheet focus	stack window
	**Show crosshair**	shows or hides the crosshair	stack window
	**Select left light sheet**	activates the light sheet on the left-hand side	Settings 1 pane
	**Select both light sheets**	activates the light sheets on both sides	Settings 1 pane
	**Select right light sheet**	activates the light sheet on the right-hand side	Settings 1 pane

Table 4.1: ImSpector software icons

5 Preparing the instrument

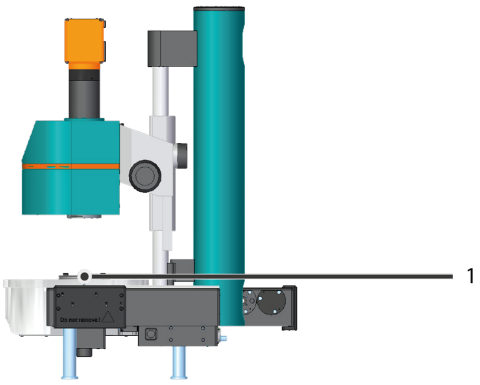
5.1 Preparing the instrument

⚠ WARNING

Contamination or infection could result in death or serious injury depending on the material used.

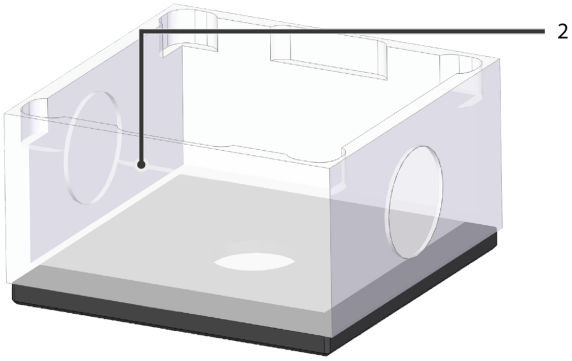
- Take care when handling samples and reagents.
- Immediately replace the waste bottle after unmounting and fasten the bottle closure to the new bottle.
- Wear personal protective equipment (such as gloves, safety glasses, etc.) as indicated in the safety data sheet for the particular substance.

1 Remove the cover plate by loosening the screw (1) using an Allen key.



2 Push the piston carefully at the bottom of the microscope chamber, and remove the cuvette by lifting it from its holders.

3 Fill the cuvette up to the notch (2) on the side with the selected imaging solution.



4 Place the cuvette securely on its holders again by holding the piston.

NOTICE! Be careful not to spill any imaging solution.

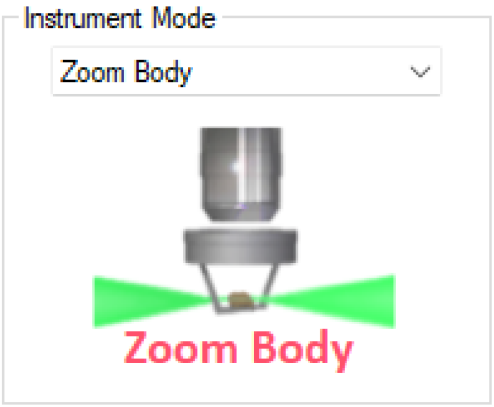
5 Lower the piston carefully until the bottom of the microscope chamber is reached.

6 Fasten the cover plate by tightening the screw.

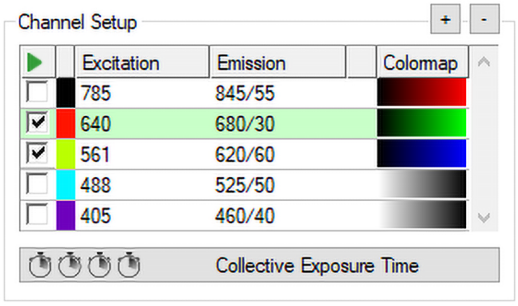
7 Attach the objective lens.

5.2 Setting the parameters for the alignment

1 Select the appropriate **Instrument Mode** in the **Measurement Wizard** for the ordered configuration, such as **Zoom Body**.

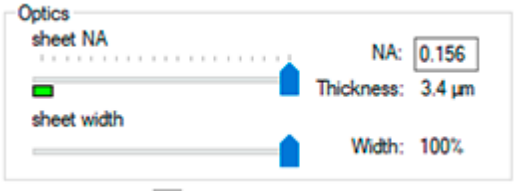


2 Select the excitation and emission wavelengths under **Channel Setup** (**Filter for Measurement** in software version 7.3 or earlier) in the **Settings 1** pane of the **Ultrall** view. For the alignment tool, the excitation is 561 nm. The selection must be highlighted to become active.



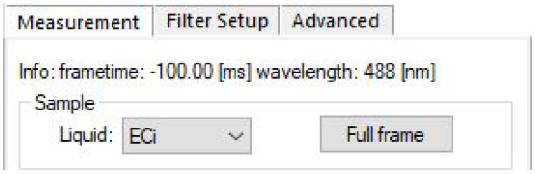
3 Set **Laser Transmission Control** between 5 and 10%.

4 Set **Optics > sheet NA** to the maximum.

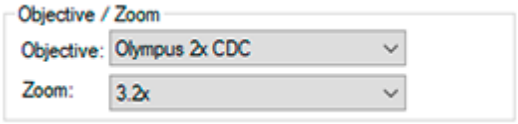


5 Set **Optics > sheet width** to 100%.

6 Select the **Measurement** tab, and select the imaging solution used in the cuvette for **Liquid**.



7 Select the objective lens, such as **LVBT 4×** with 1.0x zoom for the Super Plan configuration or **Olympus 2× CDC** with 3.2x zoom for the Zoom Body configuration. The zoom needs to be the same as set at the zoom knob.



8 Set the exposure time to a value between 100 and 200 ms in the **Camera** view.

6
Laser alignment

To generate high-contrast images with the best z-resolutions, all light sheets must be perfectly aligned to the same z-plane. A set of adjustable precision screws on the left-hand and the right-hand side of the instrument can be used to align the individual beams creating the light sheet. The Z screws enable an adjustment of the light sheets in terms of the z-plane. Screw Z1 is used for aligning the central light sheet on the left-hand side in the z-plane. Later the screw can be used for the fine adjustment of the z-shift between the left and right. The screws Z2 and Z4 are used for aligning the outer light sheets. The x-ed out screws may not be adjusted. Always start with the central light sheet before aligning the lateral light sheets.

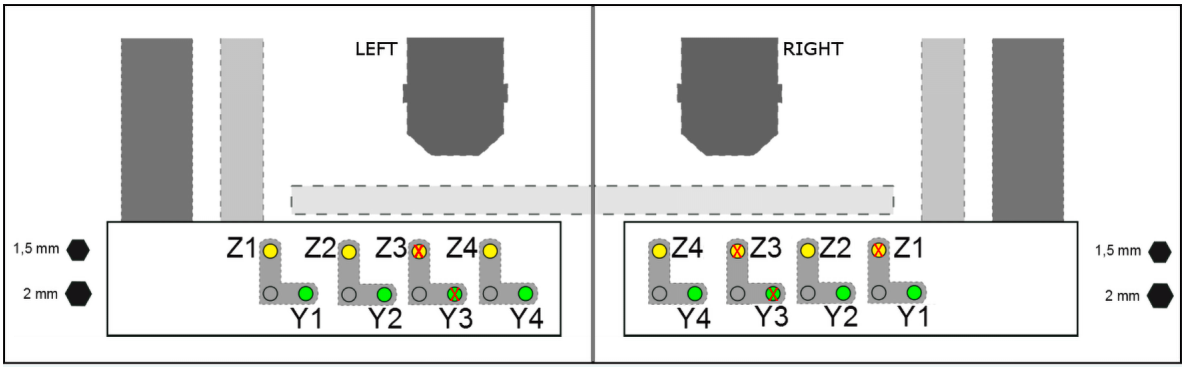


Figure 6.1: Side view of the instrument with the positions of the adjustable precision screws

6.1 Alignment tool

The alignment tool facilitates the adjustment of all light sheets. The mask carries an X with a pinhole in the center. All light sheets must pass the pinhole.

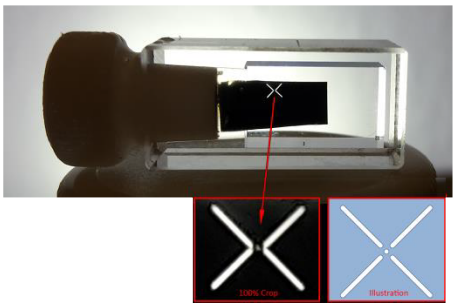
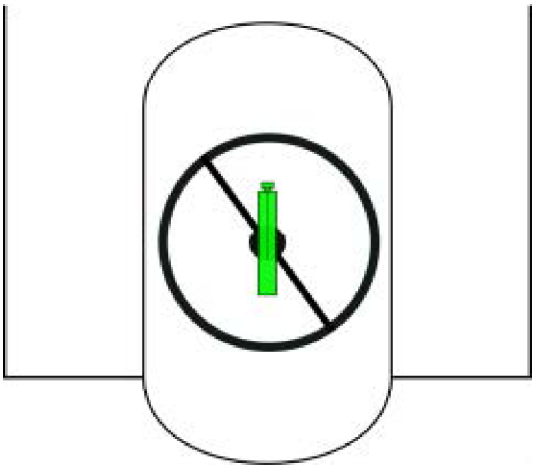


Figure 6.2: Side view of the alignment tool

6.2 Placing the alignment tool

1 Attach the alignment tool with the sample holder to the sample holder frame.



2 Place the sample holder frame into the cuvette.

6.3 Setting the light sheet path

⚠ WARNING

Direct exposure to laser beam could result in eye or even skin injury.

The instrument is equipped with a Class 3B laser product (Laser BC) or Class 4 laser product (supercontinuum WLL) according to standard IEC 60825-1:2014.

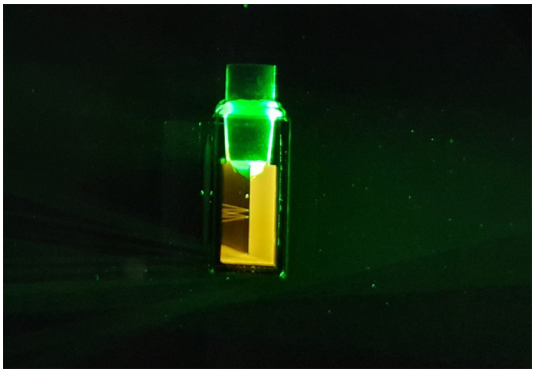
- Do not stare into laser beam.
- Do not insert reflective objects into the cuvette.
- Wear laser safety goggles.
- Do not manipulate the alignment tool during alignment.

Before setting the light sheet path, ensure that not any bubbles are present to the sides of the alignment tool.

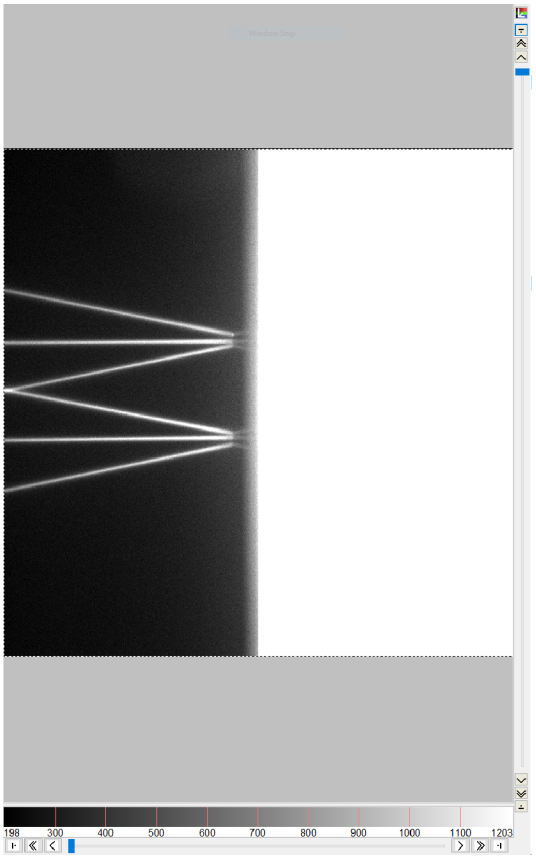
1 If necessary, mount the appropriate dipping cap.

2 Click the **Start live preview** button in the **Measurement Wizard** to start the live preview. The button turns into a yellow square.

3 Check by eye that the alignment tool is in a centered position, and move it into the light sheets by using the jog wheel. The light sheets from the right-hand side should pass the pinhole and form thin lines on the other side. The sharper the lines, the better is the z-position.



4 Lower the objective lens, and focus on the light sheets.

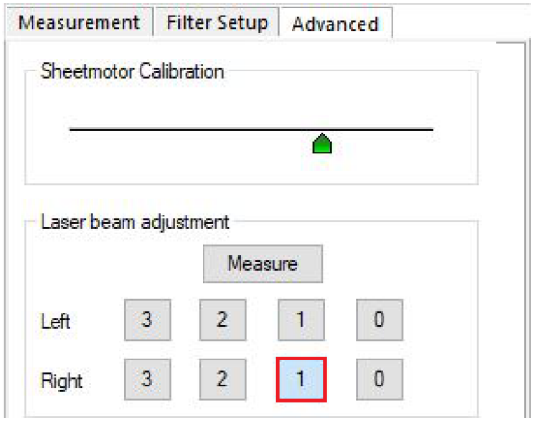


5 Click the **Show crosshair** button on the right in the stack window to activate the crosshair, and line up the alignment tool in the center.

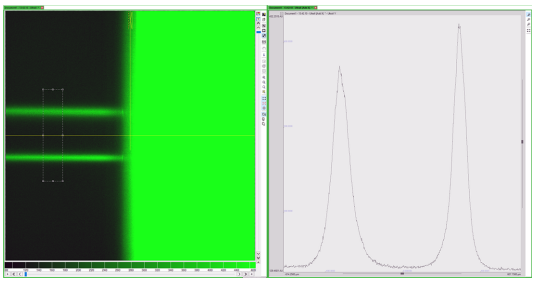
6 Place the light sheet focus indicator in the center of the field of view by clicking the **Set horizontal focus** button under the **Measurement** tab in the **Settings 1** pane.

7 Select the **Advanced** tab in the **Settings 1** pane. Always start with the central light sheet on the right-hand side. It is the reference light sheet that cannot be adjusted by the user.

8 Click **Laser beam adjustment** > **Right** > **1** to activate the central laser beam.



9 Draw a rectangle over the lines of the light sheet as the ROI, and adjust the contrast. If there are not any lines visible, move the **Sheetmotor Calibration** slider until the lines have a maximum sharpness. See **Adjusting the horizontal focus on the facing page**.



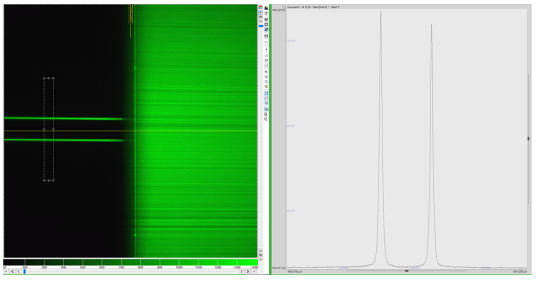
10 Right-click the ROI, and select **Add X** to add an intensity profile.

11 Right-click the dark gray part of the y-axis and select **Grow**. This setting makes it easier to visualize the intensity of the light sheet.

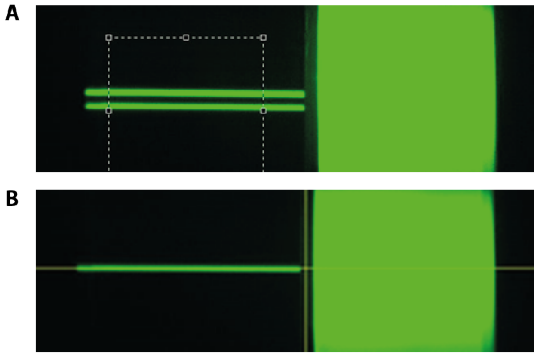
12 Use the knobs of the focusing unit:

- outer knob for the coarse focus
- inner knob for the fine focus

13 Switch between **Fit best** and **Grow** to rescale the graph and find the maximum intensity by using the **Brain** button with the mouse wheel. Keep the cursor on the button.



14 If two lines are visible passing the mask (**A**), lower or raise the alignment tool by using the mouse wheel. If the lines move closer to each other, the mouse wheel is scrolled in the correct direction. The aim is to align the pinhole located in the center of the mask to the central light sheet. This aligns all light sheets to this position (**B**).

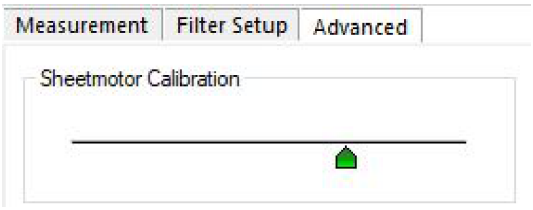


If it is not possible to align the light sheet by lowering or raising the alignment tool, the actual position of the horizontal focus does not match with the position displayed in the ImSpector software. Align the central light sheet as well as possible before adjusting the horizontal focus.

Adjusting the horizontal focus

1 Select the **Advanced** tab on the bottom of the **Settings 1** pane.

2 Move the **Sheetmotor Calibration** slider until only one line passes the pinhole. Ensure that the light sheet focus is always in the center and the **Optics > sheet NA** is at the maximum. If necessary, readjust the z-direction.

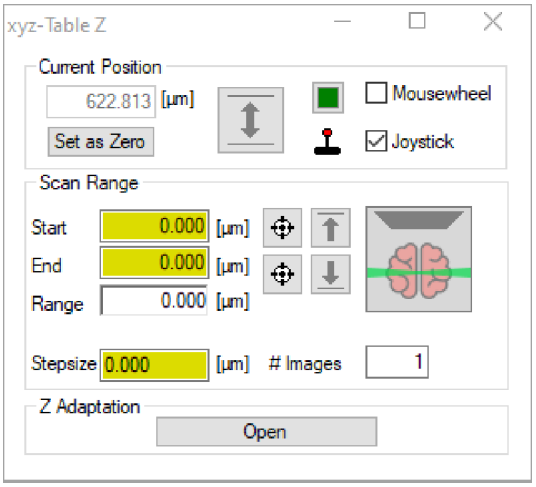


3 If only one line passes the pinhole in the center of the mask, activate the intensity profile.

4 Increase the intensity of this light sheet by switching between moving the alignment tool in z-direction and moving the slider. If not any further increase of the intensity is measurable, the pinhole is aligned to the light sheet.

5 Click **Save Settings** on the toolbar to fix the adjusted horizontal focus.

6 Click **Set as Zero** under **Current Position** in the **Settings 2** pane to set a reference point of the alignment tool.

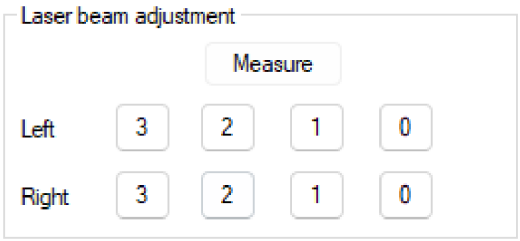


After the settings have been saved, the alignment tool must not be moved again. It is highly recommended to block the third button from the left of the jog wheel.

6.4 Adjusting the lateral light sheets on the right-hand side

To adjust the lateral light sheets on the right-hand side, the horizontal focus must not be changed.

1 Click **Laser beam adjustment** > **Right** > **3** in the **Advanced** tab in the **Settings 1** pane to activate all three laser beams.



2 Adjust the height of the lateral light sheets. Start with the rear light sheet by using the screw **Z2**. See **Figure 6.1**. Use an Allen key to align the light sheet to the pinhole.

3 Repeat the procedure with the front light sheet by using the screw **Z2**. See **Figure 6.1**.

4 Draw a ROI over the light sheets passing the pinhole in the stack window, right-click the ROI, and select **Add X**.

5 Switch between **Fit best** and **Grow** to rescale the graph.

6 Check the adjustment by clicking the **Brain** button under **Scan Range** in the **Settings 2** pane. Keep the cursor on the button, and scroll with the mouse wheel to move the alignment tool up and down.
If the intensity peaks decrease simultaneously, the alignment is sufficient.

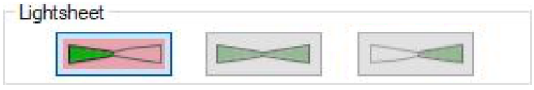
7 To move back the alignment tool to the reference position, regardless whether the adjustment was successful, type **0** under **Current Position**.

8 Press the **Move to set position** button.
Alternatively use the mouse wheel.

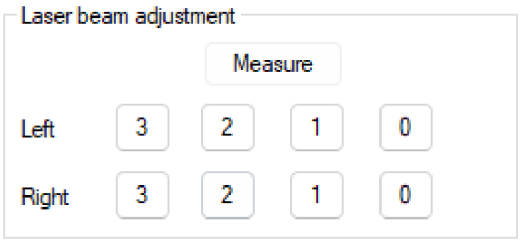
Proceed with adjusting the central light sheet on the left-hand side.

6.5 Adjusting the central light sheet on the left-hand side

1 Click **Lightsheet** > **Select left lightsheet** in the **Settings 1** pane.



2 Click **Laser beam adjustment** > **Left** > **1** in the **Advanced** tab to activate the central laser beam.



3 Draw a rectangle over the illuminated part and the lines of the light sheet as the ROI, and adjust the contrast.

4 Align the central light sheet of the left side to the pinhole by using the screw Z1 on the left-hand side.

5 Move the **Sheetmotor Calibration** slider in the **Settings 1** pane until only one light sheet passes the pinhole.

6 Ensure that the slider for the horizontal focus is still in the center and the NA is at maximum. If necessary, adjust the position.

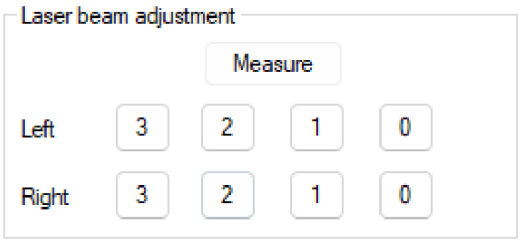
7 Repeat moving the screw in the z-direction and adjusting the position of the horizontal focus to find the maximum intensity.

8 If it is not possible to make only one light sheet pass the pinhole, observe the instructions under **Adjusting the horizontal focus on page 33**.

6.6 Adjusting the lateral light sheets on the left-hand side

To adjust the lateral light sheets on the left-hand side, the horizontal focus must not be changed.

1 Click **Laser beam adjustment** > **Left** > **3** in the **Advanced** tab in the **Settings 1** pane to activate all three laser beams.



2 Adjust the height of the lateral light sheets. Start with the rear light sheet by using the screw **Z2**. See **Figure 6.1**. Use an Allen key to align the light sheet to the pinhole.

3 Repeat the procedure with the front light sheet by using the screw **Z2**. See **Figure 6.1**.

4 Draw a region of interest over the light sheets passing the pinhole in the stack window, and activate the intensity profile.

5 Switch between **Fit best** and **Grow** to rescale the graph.

6 Check the adjustment by clicking the **Brain** button under **Scan Range** in the **Settings 2** pane. Keep the cursor on the button, and scroll with the mouse wheel to move the alignment tool up and down.
If the intensity peaks decrease in the same way, the alignment is sufficient.

7 To move back the alignment tool to the reference position, regardless whether the adjustment was successful, type **0** under **Current Position**.

8 Press the **Move to set position** button.
Alternatively use the mouse wheel.

If the alignment from the right-hand side to the left-hand side is not successful, check the position of the alignment tool again.

If the alignment is successful, click the **Start Live Preview** button again to stop the laser activity.

7 Setting up an experiment

7.1 Placing the sample

1 Fix the sample to the sample holder of the sample holder frame.

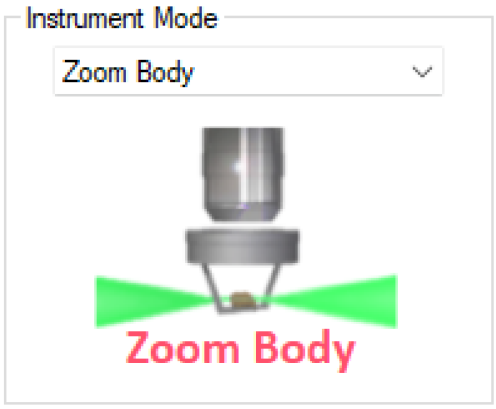
2 Attach the sample holder to the sample holder frame.

3 Place the sample holder frame into the cuvette.

NOTICE! Ensure that no medium gets into the microscope chamber.

7.2 Instrument Mode

1 Select the appropriate **Instrument Mode** in the **Measurement Wizard** for the ordered configuration, such as **Zoom Body**.



All instrument modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See **Technical support on page 53**.

7.3 Measurement Mode

In the **Measurement Mode** list, you can select different predefined measurement modes.

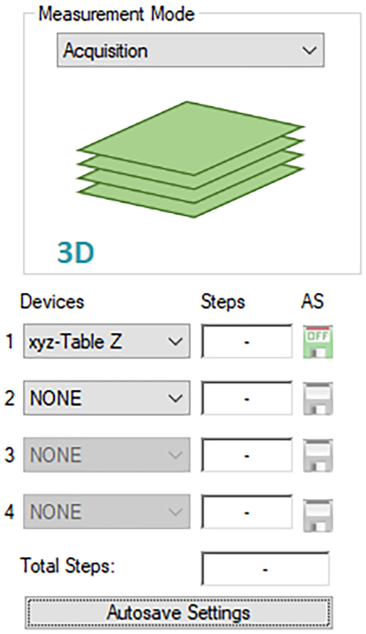


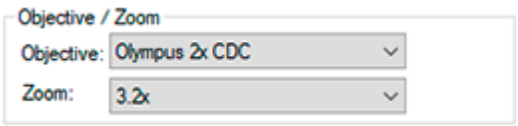
Figure 7.1: Measurement Mode list

All measurement modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See **Technical support on page 53**.

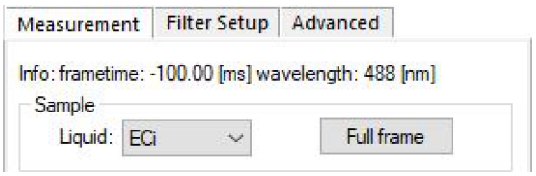
7.3.1 Aligning the horizontal focus on the sample

The alignment tool does not have exactly the same optical properties as a sample. Thus, the position of the horizontal focus must always be checked on the sample. This is also the case when changing objective lenses, as the FOV may differ slightly.

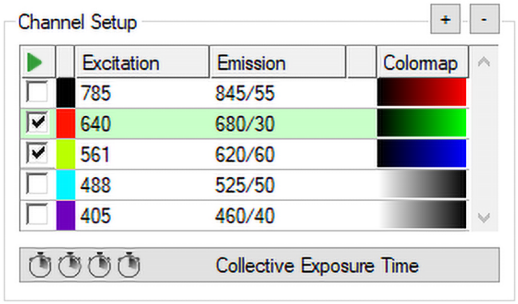
1 Under **Objective / Zoom**, select the appropriate objective lens and the **Zoom** in the **Settings 1** pane.



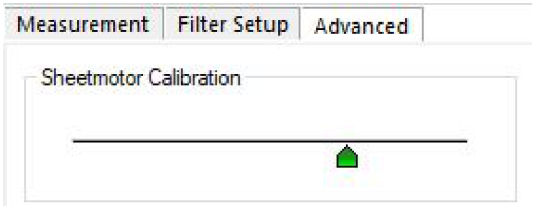
2 Click the **Measurement** tab, and select the imaging solution used in the cuvette for **Liquid**.



3 Under **Channel Setup**, select the excitation and emission wavelengths for your sample.



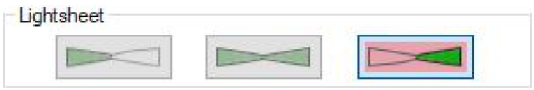
4 To set the current excitation wavelength as the new reference wavelength, click the **Save** button under **Chromatic Correction** in the **Advanced** tab.



5 Move the slider under **Laser Transmission Control** to set the laser power.

NOTICE! Higher laser power bleaches the sample faster.

6 Click **Lightsheet > Select right lightsheet**.



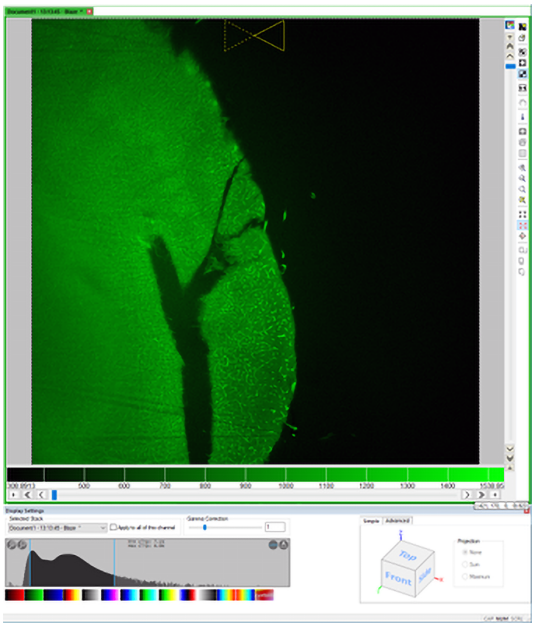
7 Click the **Start live preview** button in the **Measurement Wizard** to start the live preview.

8 Lower the objective lens.

9 Use the jog wheel to move the sample inside the cuvette until the surface of the sample is visible in the FOV.

10 Focus on the sample.

11 Place the light sheet focus indicator on the sample, and check if it corresponds to the actual position of the light sheet focus. Move the **Sheetmotor Calibration** slider.

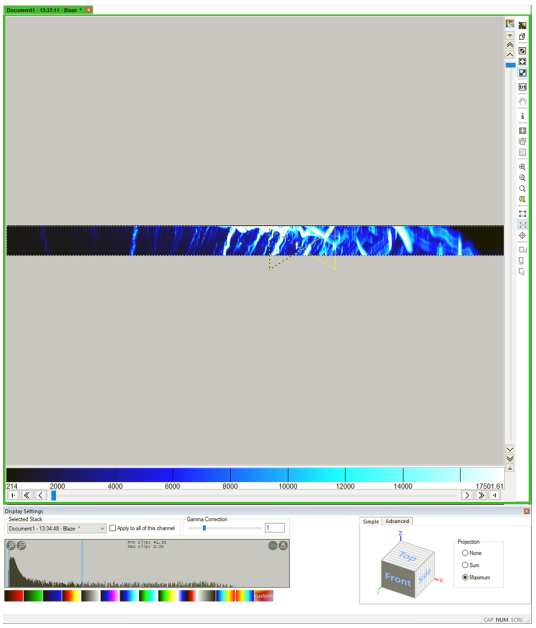


12 Move the light sheet focus indicator along the sample to check if the contrast is always best below.

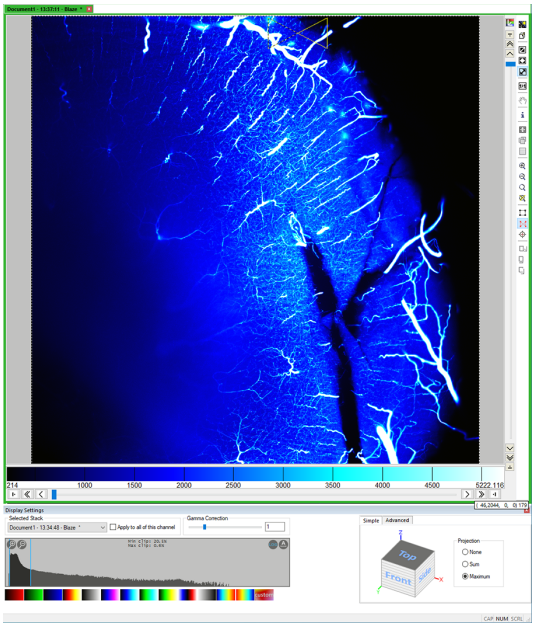
13 Set up a z-stack of about 200 micrometers with two micrometers step size to confirm the calibration.

14 Select **Measurement Mode > Acquisition** and deactivate autosave.

15 Open **Display settings** and perform a maximum projection of the front view. Check if the region with the best z-resolution corresponds to the indicated light sheet focus position. If necessary, move the **Sheetmotor Calibration** slider.



16 Perform a maximum projection of the top view. Check if the region with the best contrast corresponds to the indicated light sheet focus position. If necessary, move the **Sheetmotor Calibration** slider.



17 If the alignment is satisfactory, repeat the steps for the left light sheet.

18 Click **Save Settings** on the toolbar.

7.3.2 Correcting the chromatic offset of the horizontal focus

When using different wavelengths, the horizontal focus of each channel is in a different position compared to the reference wavelength defined in the **Measurement** tab. These chromatic offsets in the excitation path have to be corrected using a sample.

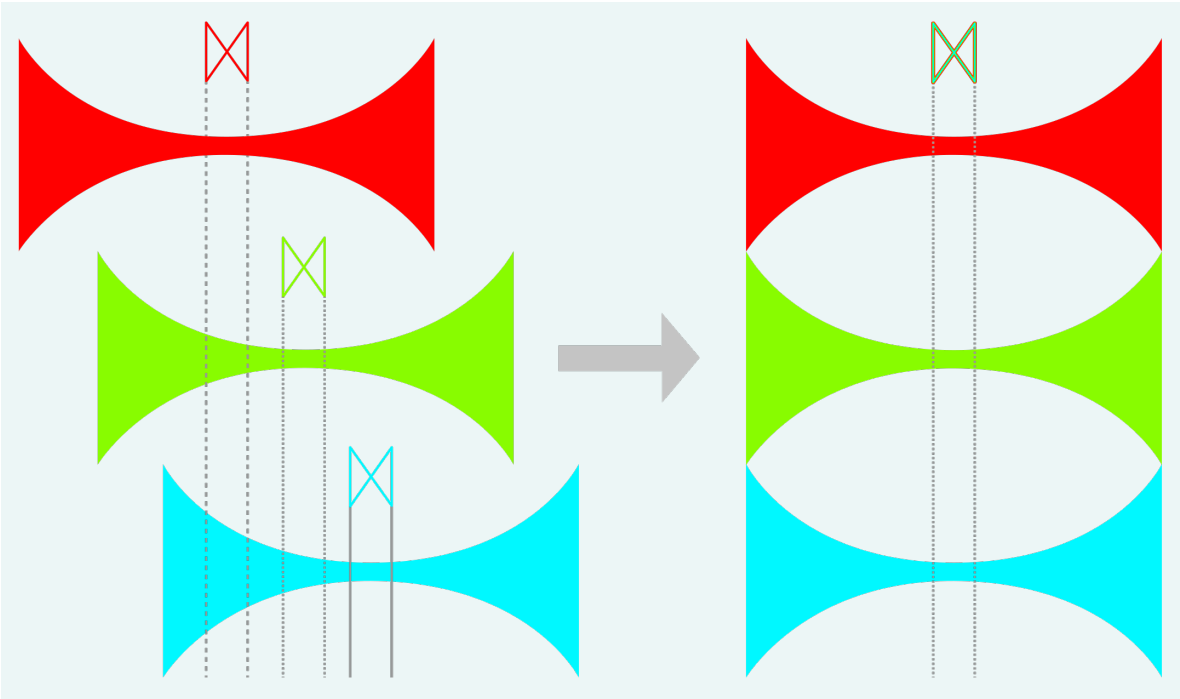
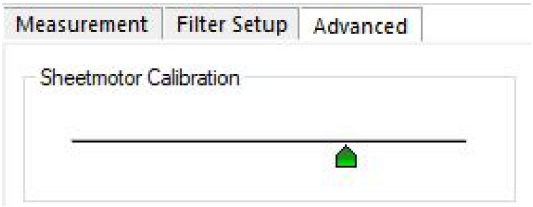


Figure 7.2: Chromatic offsets

1 Select the channel to be corrected.

2 Switch to the live preview, and focus on the sample.

3 Place the light sheet focus indicator on the sample, and check if it corresponds to the actual position of the light sheet focus. Move the **Sheetmotor Calibration** slider.



4 Move the light sheet focus indicator along the sample to check if the contrast is always best below.

5 Optional: Capture a z-stack without autosave as described under **Aligning the horizontal focus on the sample on page 38**. If necessary, move the **Sheetmotor Calibration** slider.

6 Repeat the chromatic correction for the left light sheet.

7 Click **Save Settings** on the toolbar.

7.3.3 Capturing an image

1 Move in the z-direction to the starting position.

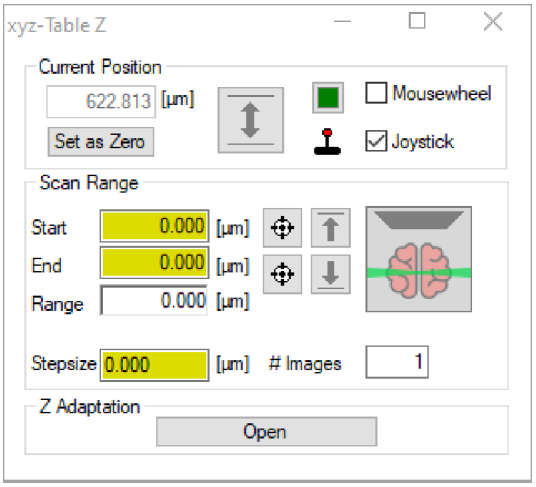
2 Click **Current Position** > **Set as Zero** in the **Settings 2** pane.

3 Click the **Take current position as start** button under **Scan Range** > **Start**.

4 Move in the z-direction to the end position.

5 Click the **Take current position as end** button under **Scan Range** > **End**.

6 Define the increment by entering it under **Scan Range** > **Stepsize**.

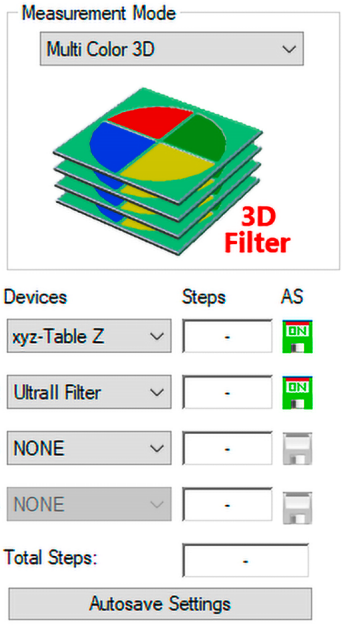


7 Check the **Autosave Settings** in the **Measurement Wizard** again.

8 Click the **Start measurement** button to start the measurement.

7.3.4 Capturing a multicolor z-stack

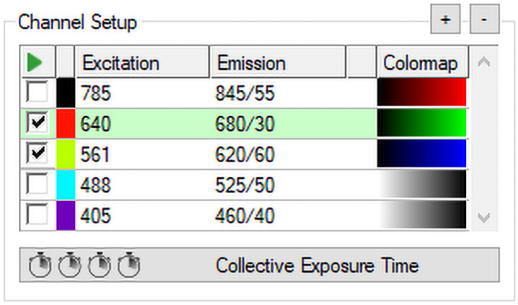
1 Select **Measurement Mode** > **Multi Color 3D** in the **Measurement Wizard**.



2 Select **xyz-Table Z** and **Ultrall Filter** in the **Devices** list.

3 Click the **Autosave** button at the end of the row under **AS**. The button turns green.

4 Select the correct excitation and emission wavelengths for your sample under **Channel Setup** (**Filter for Measurement** in software version 7.3 or earlier) in the **Settings 1** pane. The selected checkboxes are used.



5 Move the slider under **Laser Transmission Control** while the filter is highlighted to set the laser power for each individual filter.

NOTICE! Higher laser power bleaches the sample faster.

6 Select the shortest emission wavelength, and create a sharp picture.

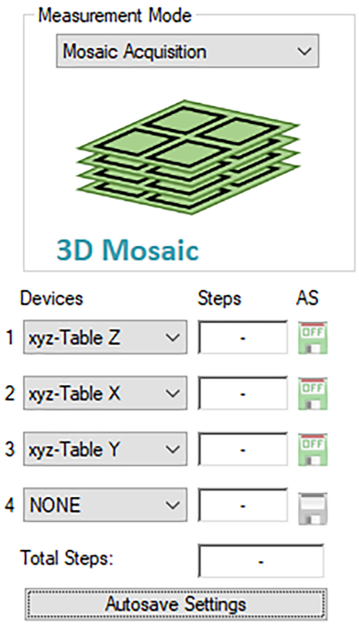
7 Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction** button, starting with the next longer wavelength.

8 If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.

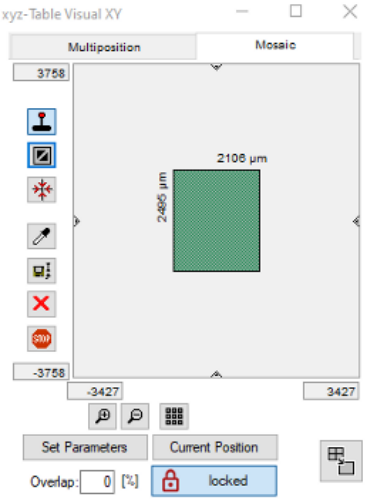
9 To start the measurement, proceed as described under **Capturing an image on the previous page**.

7.3.5 Capturing a mosaic image

1 Select **Measurement Mode** > **Mosaic Acquisition** in the **Measurement Wizard**.



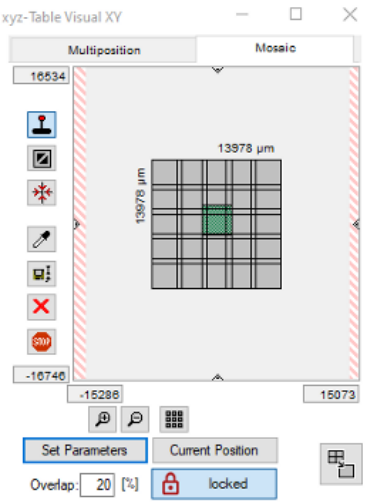
2 Click the **Mosaic** tab in the **xyz-Table Visual XY** view.



3 Double-click the green square representing the field of view. The square turns gray.

4 Click and drag the black dots at the edges to create a mosaic.

5 Enter a percentage value to set an **Overlap**.



6 To start the measurement, proceed as described under **Capturing an image on page 42**.

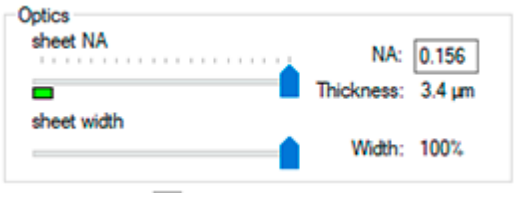
7.4 Optimizing the measurement settings

1 Draw a rectangle over an illuminated and/or a dark region. If drawing is not possible, click the **ROI for active profile** button on the right in the stack window twice.

2 Click the **Adjust window/level** button to achieve a better contrast.

3 Click the **Rayleigh ROI** button to show the horizontal focus. Move the horizontal focus in the picture until you have the optimal contrast for your region of interest.

4 Optimal: Move the slider under **Optics > sheet NA** to reduce the numerical aperture of the light sheet. This creates a more homogeneous light sheet, but reduces the resolution in the z-plane.



5 Optional: Move the slider under **Optics > sheet width** to change the sheet width of the light sheet. This results in more light being focused on a smaller region of the sample.

The settings described may need to be adjusted when the zoom settings are changed.

8 Maintenance

8.1 Servicing

Service the instrument every 12 months. Contact Miltenyi Biotec for support.

8.2 Cleaning the instrument

NOTICE

Property damage.

Wrong cleaning could damage sensitive equipment.

- Use only small amounts of suitable cleaning agents on a soft cloth to wipe the instrument.

1. Unplug the instrument.
2. To disinfect, wipe accessible surfaces with 70% ethanol or isopropanol.
3. If necessary, wipe the instrument with a damp tissue.

8.3 Cleaning the cuvette

Before cleaning the cuvette, dispose of the imaging solution in accordance with local regulations.

1. Clean the cuvette with a cleaning solution suitable for the imaging solution used.

NOTICE! Be careful not to damage the windows of the cuvette.

9 Technical data and specifications

9.1 Technical data

The safety of the instrument may be compromised if used outside its specifications.

UltraMicroscope II Imaging System		
General information	**Zoom Body configuration**	**Super Plan configuration**
dimensions (w × d × h)	54 cm × 65 cm × 82 cm	54 cm × 65 cm × 73 cm
weight	47 kg (without laser source)	
nominal input voltage	12 V DC, provided by external power adapter	
Working conditions		
temperature	15–27 °C	
humidity	max. 70% relative humidity, noncondensing	
Storage conditions		
temperature	4–55 °C	
humidity	max 70% relative humidity, noncondensing	
Transport conditions		
temperature	4–55 °C	
humidity	max 70% relative humidity, noncondensing	
Cuvette		
imaging solutions	aqueous buffers or organic solvents	
sample travel range (x, y, z)	10 mm, 10 mm, 10 mm	
cuvette size	72 mm × 74 mm × 35 mm	
refraction index matching	1.33–1.56	

UltraMicroscope II Imaging System				
Detection optics	**Zoom Body configuration**	**Super Plan configuration**		
objective lens	2×	1.1×	4×	12×
zoom | postmagnification	0.63×–6.3×	0.6×–2.5×		
resolution at detector	0.52–5.16 µm	4.8–19.6 µm	1.3–5.4 µm	0.44–1.8 µm
numerical aperture	0.5	0.1	0.35	0.53
working distance	5.6 mm	≤17 mm	≤16 mm	≤10.9 mm
emission filters	seven filters, Ø 32 mm	seven filters, Ø 43 mm		
chromatic correction	dynamic 400–850 nm			
Sheet optics				
illumination	uni- and bidirectional			
number of light sheets	1–6			
thickness	4–24 µm			
width	1–20 mm			
numerical aperture	0.0135–0.135			
focus positioning	dynamic			
Camera specifications				
detector	4.2 Mpx sCMOS camera			
active pixels (w × h)	2048 × 2048			
pixel size	6.5 µm × 6.5 µm			
sensor size	13.3 mm × 13.3 mm; 18.8 diagonal			
readout noise	0.8 med e^-			
maximal frame rates	100 fps			
maximum quantum efficiency	82%			

Table 9.1: Technical data of the instrument

9.2 UK Declaration of Conformity

This declaration of conformity is issued under the sole responsibility of the manufacturer:

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

This declaration relates exclusively to the machinery in the state in which it was placed on the market, and excludes components which are added and/or operations carried out subsequently by the final user.

The declaration of conformity refers to the product identified as follows:

Description: Laboratory equipment
Model: UltraMicroscope II Imaging System

The machinery complies with all essential requirements of the following legislations:

Supply of Machinery (Safety) Regulations 2008
The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations 2012
Electromagnetic Compatibility Regulations 2016

The machinery is in conformity with the following UK designated standards:

EN ISO 12100:2010
EN 60825-1:2014
EN 61010-1:2010

Person authorized to compile the relevant technical documentation:

Winfried Brempel
Miltenyi Biotec B.V. & Co. KG
Astastraße 14
33617 Bielefeld
Germany

9.3 EC/EU Declaration of Conformity

This declaration of conformity is issued under the sole responsibility of the manufacturer:

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

This declaration relates exclusively to the machinery in the state in which it was placed on the market, and excludes components which are added and/or operations carried out subsequently by the final user.

The declaration of conformity refers to the product identified as follows:

Description: Laboratory equipment
Model: UltraMicroscope II Imaging System

The machinery complies with all essential requirements of the following directives:

2006/42/EC Machinery
2011/65/EU Restriction of the use of certain hazardous substances in electrical & electronic equipment
2014/30/EU Electromagnetic compatibility

The machinery is in conformity with the following harmonized standards:

EN ISO 12100:2010
EN 60825-1:2014
EN 61010-1:2010

Person authorized to compile the relevant technical documentation:

Winfried Brempel
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10 Technical support

For technical support, contact your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support at Miltenyi Biotec headquarters:

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technicalsupport@miltenyi.com

Visit **www.miltenyibiotec.com** for local Miltenyi Biotec Technical Support contact information.

11 Legal notes

11.1 Limited warranty

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Miltenyi Biotec warrants that this product will operate or perform substantially in conformance with Miltenyi Biotec's published specifications and be free from material defects in material and workmanship, when subjected to normal, proper, and intended usage by properly trained personnel, for the period of time set forth in the product documentation or package inserts accompanying the product (the "Warranty Period").

Miltenyi Biotec agrees, during the Warranty Period, to repair or replace, at Miltenyi Biotec's option, the defective product so as to cause the same to operate in substantial conformance with said published specifications; provided that you shall (a) promptly notify Miltenyi Biotec in writing upon the discovery of any nonconformity or defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim; and (b) return the nonconforming or defective product to Miltenyi Biotec, freight prepaid, only after receipt of a Return Material Authorization ("RMA") from Miltenyi Biotec, which may include biohazard decontamination procedures and other product-specific handling instructions, if applicable.

Miltenyi Biotec shall have no obligation to make repairs, replacements, or corrections to the product or any component thereof required, in whole or in part, as the result of (i) normal wear and tear, (ii) improper handling, installation, operation, storage, service, maintenance, or repair, (iii) failure to follow the instructions, cautions, warnings, and notes set forth in the product documentation provided with the product or provided by Miltenyi Biotec from time to time, (iv) abnormal use, misuse, neglect, abuse, mishandling, misapplication, modification, or alteration of the product, (v) use of the product in a manner for which it was not designed, (vi) causes external to the product such as, but not limited to, power failure or electrical power surges, (vii) use of the product in combination with equipment, accessories, consumables, or software not supplied or approved by Miltenyi Biotec, or (viii) accident, disaster, or acts of God. ANY INSTALLATION, MAINTENANCE, REPAIR, SERVICE, OR ALTERATION TO OR OF, OR OTHER TAMPERING WITH, THE PRODUCT PERFORMED BY ANY PERSON OR ENTITY OTHER THAN MILTENYI BIOTEC AUTHORIZED PERSONNEL WITHOUT MILTENYI BIOTEC'S PRIOR WRITTEN APPROVAL, OR ANY USE OF REPLACEMENT PARTS NOT SUPPLIED BY MILTENYI BIOTEC, SHALL IMMEDIATELY VOID AND CANCEL ALL WARRANTIES WITH RESPECT TO THE AFFECTED PRODUCT.

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