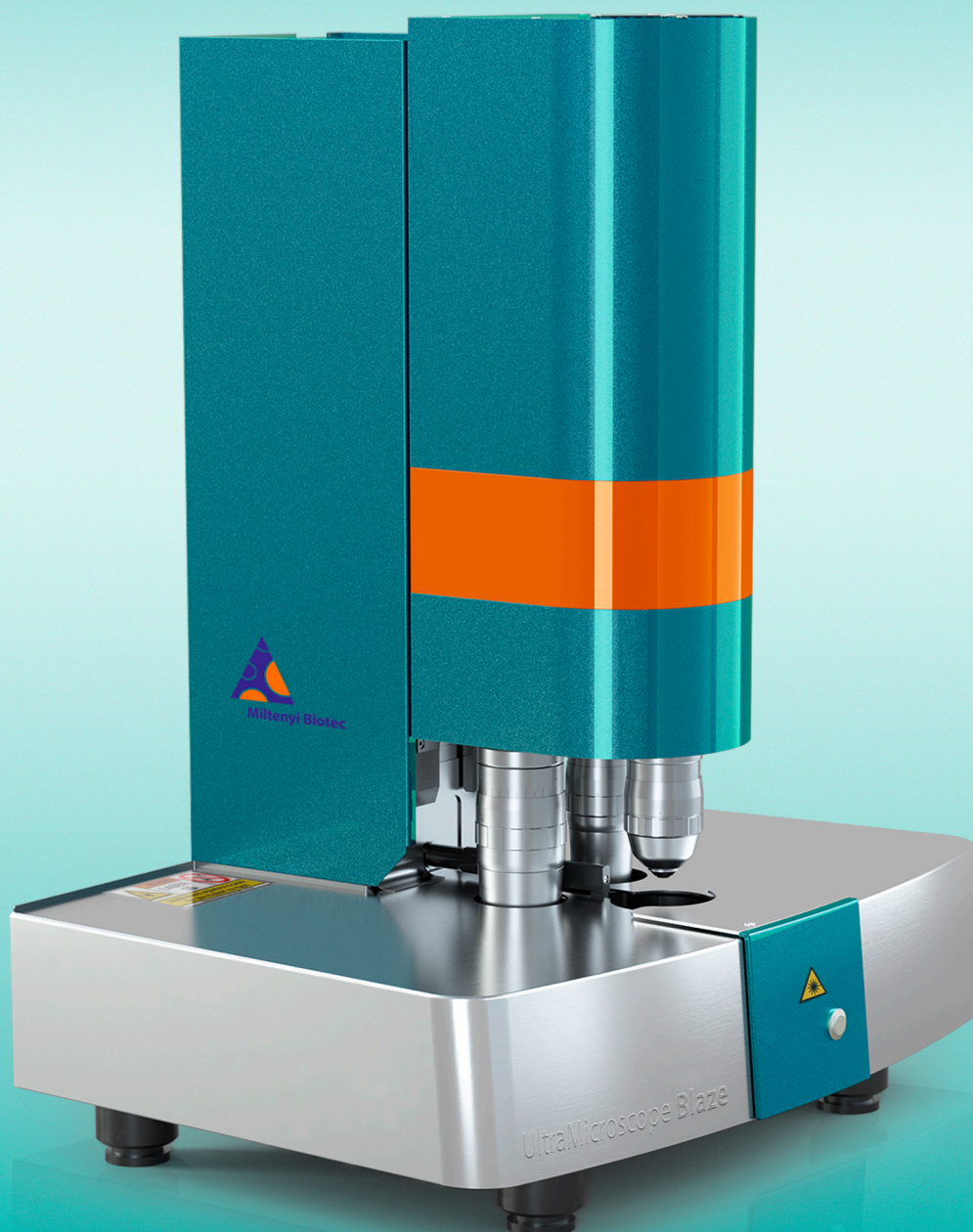


Miltenyi Biotec

UltraMicroscope Blaze™ Imaging System

User manual



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UltraMicroscope Blaze™ Imaging System

User manual

Original instructions

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1.1 Read the user manual first

WARNING

Before using the UltraMicroscope Blaze 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 shown 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.

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



CRUSHING OF HANDS HAZARD



LASER RADIATION HAZARD



WEEE (WASTE 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



UNITED KINGDOM CONFORMITY ASSESSED MARKING

Safety labels

Pay attention to the hazard zones 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.

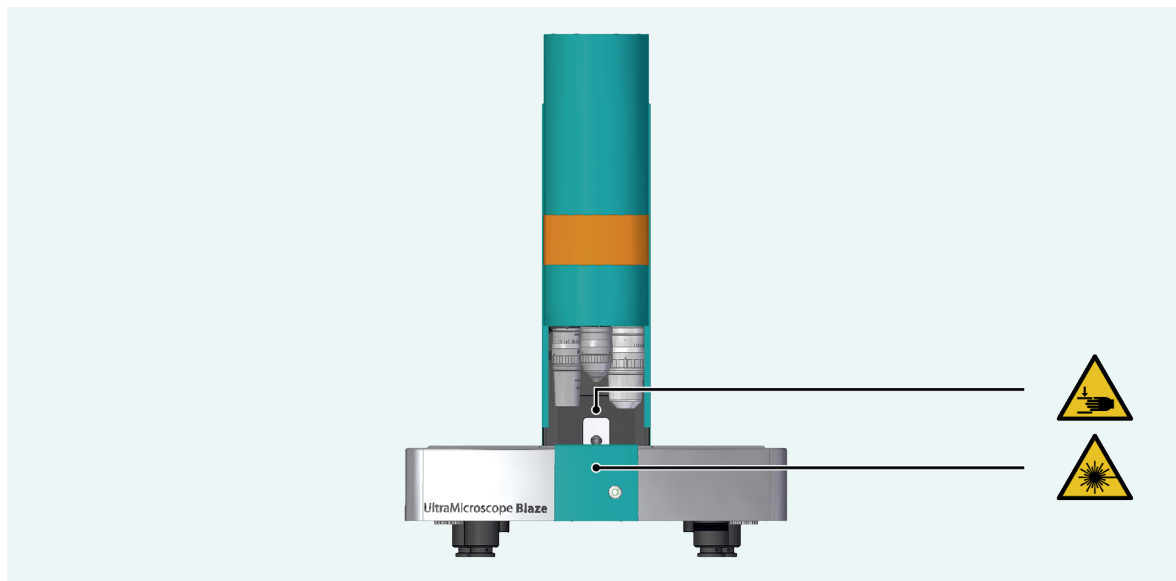


Figure 2.1: Hazard zones and safety symbols on the front side of the instrument

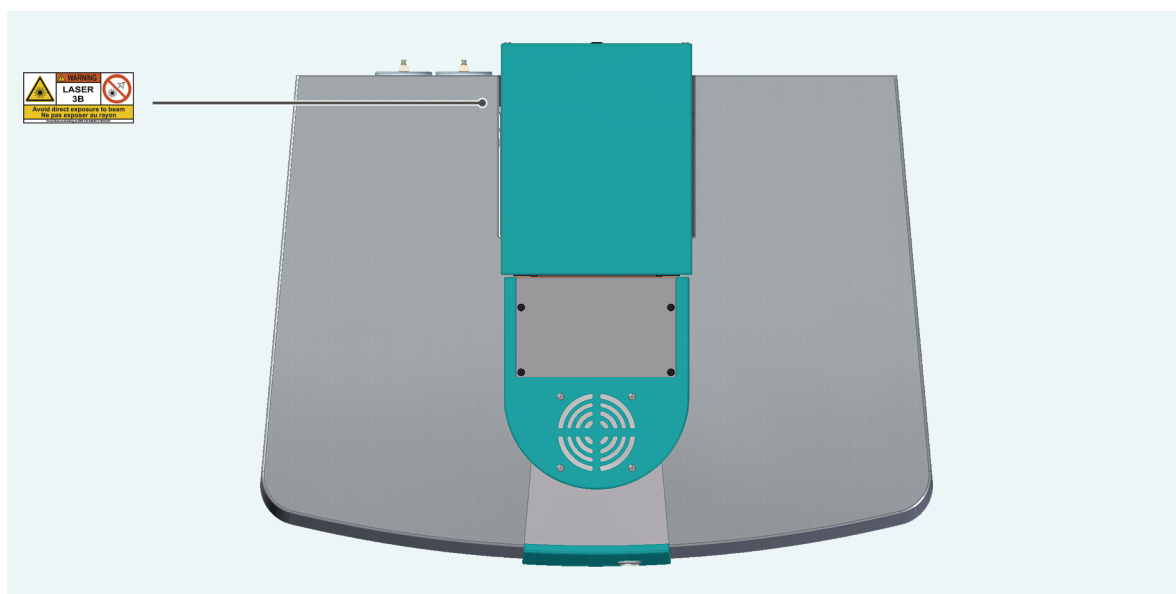


Figure 2.2: Hazard zones and safety symbols on the top side of the instrument

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.

Automated detection unit

The automated detection unit moves while the instrument is in operation.

- Keep away from the automated detection unit while the instrument is in operation.
- Do not obstruct the movement of the automated detection unit.

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 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.

3.1 The UltraMicroscope Blaze 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.

The UltraMicroscope Blaze Imaging System enables seamless switching between different objectives and magnification lenses with the click of a button while keeping images sharp with the autofocus feature. Automated movement of the cuvette facilitates sample loading and exchange.

The UltraMicroscope Blaze Imaging System uses cutting-edge illumination optics to slightly tilt 2×3 bidirectional light sheets, with their Rayleigh lengths overlapping in the entire field of view. 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 automated detection unit can switch automatically between the MI Plan objective lenses.

The UltraMicroscope Blaze Imaging System can be upgraded with an XXL cuvette, which makes imaging of larger samples as big as a human kidney or a whole mouse model possible.

3.2 Intended use

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

3.3 Instrument description

3.3.1 Front view

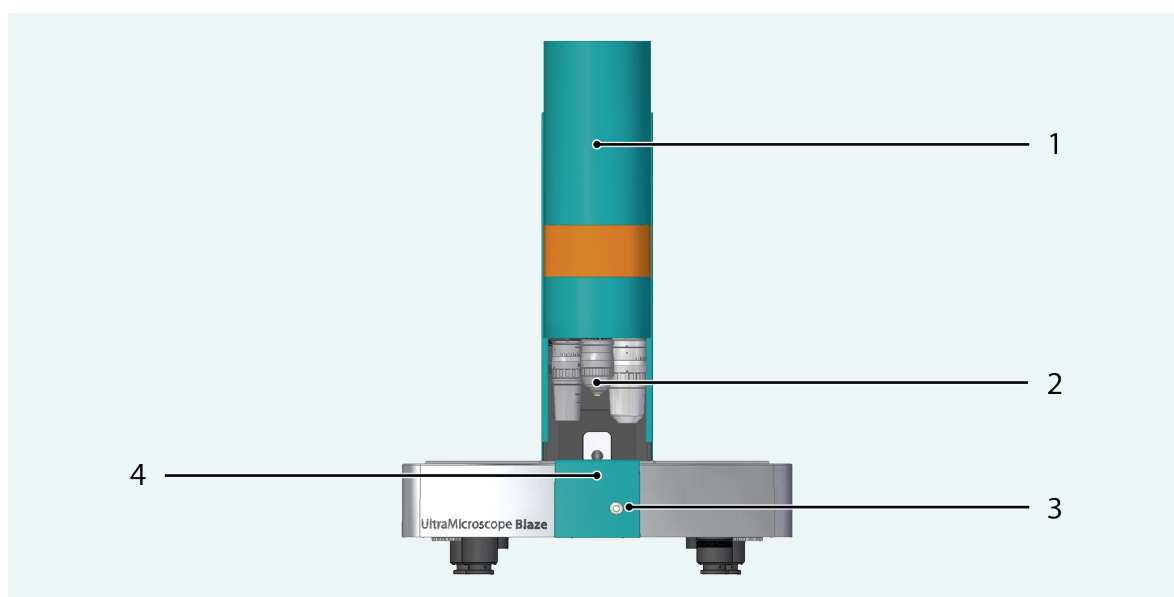


Figure 3.1: Front view of the instrument

- | | | | |
|----------|--------------------------|----------|--------------------|
| 1 | Automated detection unit | 3 | Automated drawer |
| 2 | Objective lenses | 4 | Microscope chamber |

3.3.2 Rear view

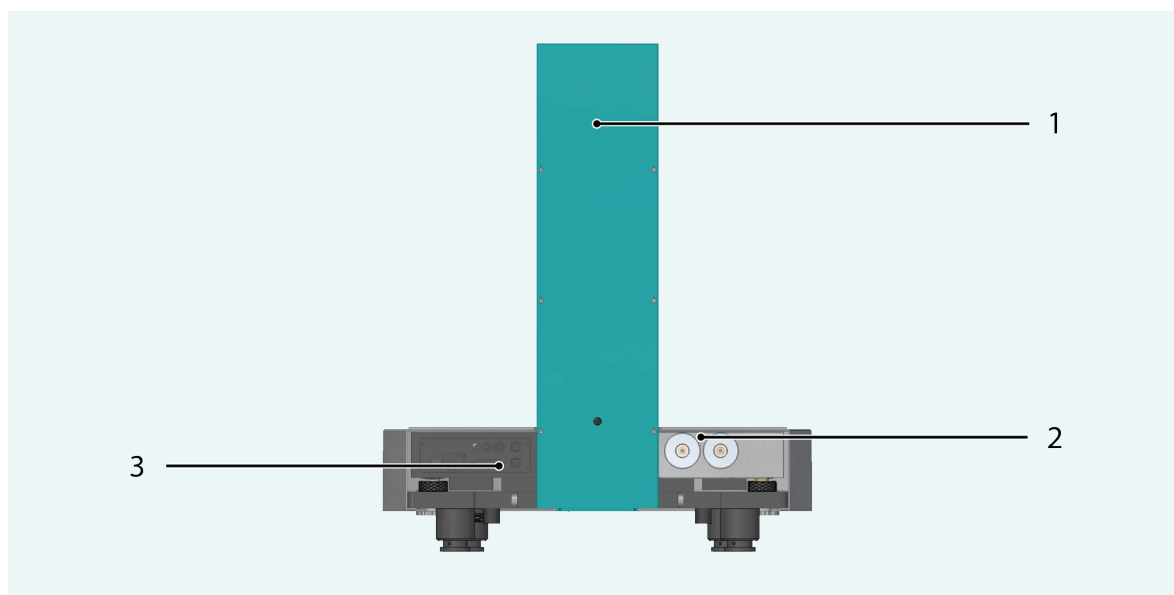


Figure 3.2: Rear view of the instrument

- | | | | |
|----------|------------------------|----------|-----------|
| 1 | Type label | 3 | I/O ports |
| 2 | Laser fiber connection | | |

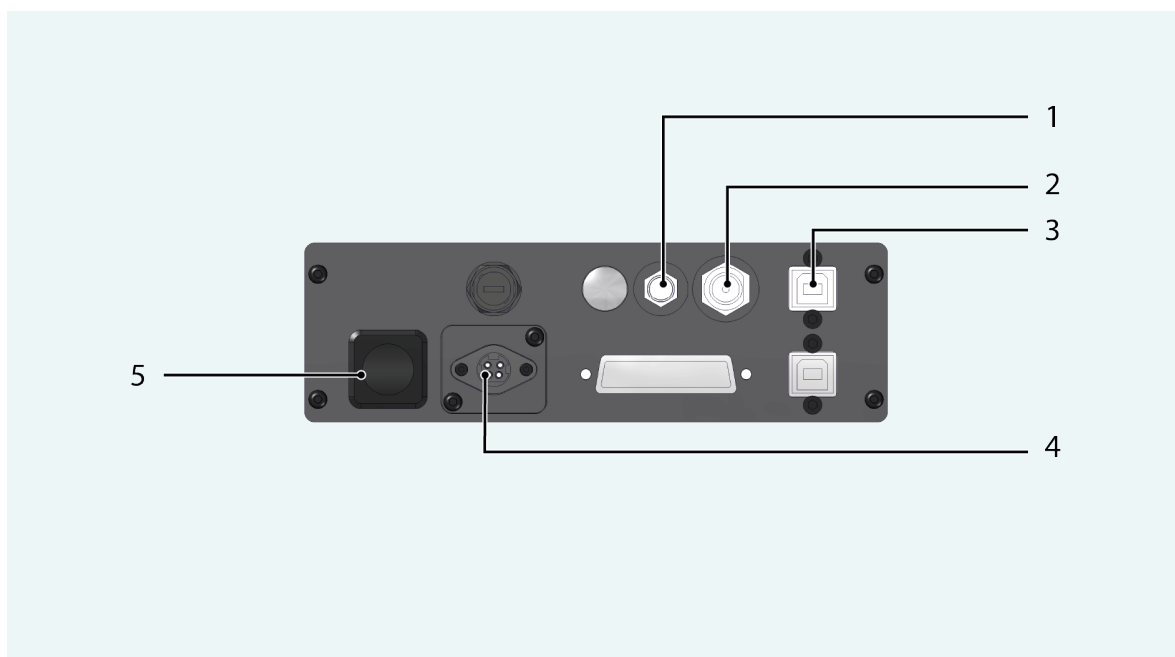


Figure 3.3: Detail view of the I/O ports and power switch

- | | | | |
|---|----------------|---|--------------|
| 1 | Jog wheel | 4 | Power port |
| 2 | Camera trigger | 5 | Power switch |
| 3 | PC connection | | |

3.3.3 Equipment

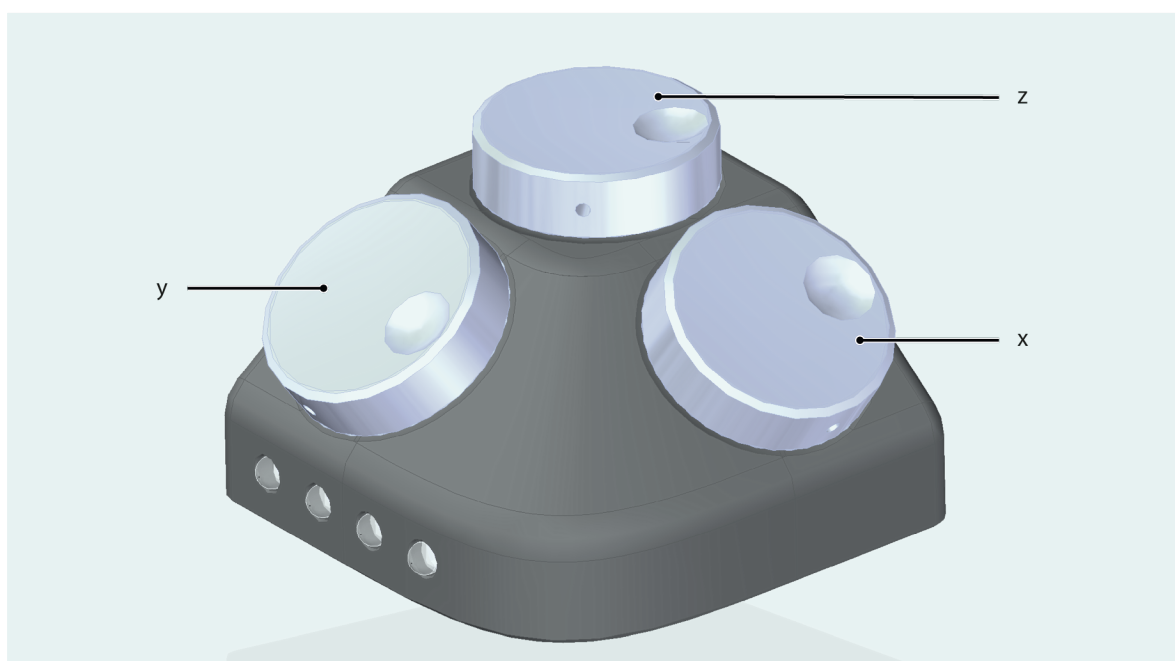


Figure 3.4: Jog wheel for moving the sample arm in three directions (x, y, z) and sensitivity

4

Installation

4.1 Components

The Beam Combiner (BC) and the White Light Laser (WLL) variant 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. For details, visit www.miltenyibiotec.com, or contact your local sales representative.

Components included in the delivery

UltraMicroscope Blaze Imaging System

Cuvette

Sample holder set

Jog wheel

Power adapter for instrument

Acquisition workstation with preinstalled software

Keyboard and mouse

Headset

HD webcam

Monitor

Table 4.1: Components included in the delivery

4.2 Consumables

Product name	Order no.
MACS Clearing Kit	130-126-719
MACS Deep Clearing Kit	130-136-470
MACS Imaging Solution	130-126-335

Table 4.2: Consumables that have to be ordered separately

4.3 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.

5

Operation

5.1 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 the manufacturer documentation.
- 3 Switch on the instrument. The power switch is located on the rear side of the instrument. See **Rear view of the instrument on page 16**.
- 4 Switch on the acquisition workstation, and sign in to the Windows system.
- 5 Open the ImSpector software. The switched on devices are registered and checked.

5.2 Switching off the instrument

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

5.3 Graphical user interface

	Name	Function	Location
	Save Settings	Saves all settings	Toolbar
	Start Measurement	Starts the defined measurement	Measurement Wizard
	Start Batch Measurement	Starts the defined batch measurement	Measurement Wizard
	Start Live Preview	Starts a live preview	Measurement Wizard
	Autosave	Activates autosave for the selected device	Measurement Wizard
	Auto Contrast	Adjusts the contrast of the colormap automatically to the minimum and maximum pixel value within the whole image or a shown region of interest	Stack window

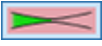
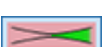
	Name	Function	Location
	ROI for Active Profile	Shows or hides the region of interest (ROI)	Stack window
	Rayleigh ROI	Switches on the light sheet focus	Stack window
	Show Crosshair	Shows or hides the crosshair	Stack window
	Move Detection Unit Down	Moves down the detection unit	Settings 1 pane
	Move Detection Unit Up	Moves the detection unit to the home position	Settings 1 pane
	Single Channel Autofocus	Focuses automatically on current channel	Settings 1 pane
	Manual Focus	Focuses manually on current channel	Settings 1 pane
	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
	Enable only the central beam	Activates only the central laser beam	Settings 1 pane
	Disable the central beam	Deactivates only the central laser beam	Settings 1 pane
	Enable all three beams	Activates all laser beams	Settings 1 pane

Table 5.1: ImSpector software icons

6

Preparing the instrument

This chapter describes the preparation of the instrument. This includes exchanging the imaging solution and setting the parameters for the alignment.

6.1 Exchanging the imaging solution

If the imaging solution has to be exchanged, it is necessary to remove the cuvette.

⚠ WARNING

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

- Take care when handling samples and reagents.
- Wear personal protective equipment (such as gloves, safety glasses, etc.) as indicated in the safety data sheet for the particular substance.

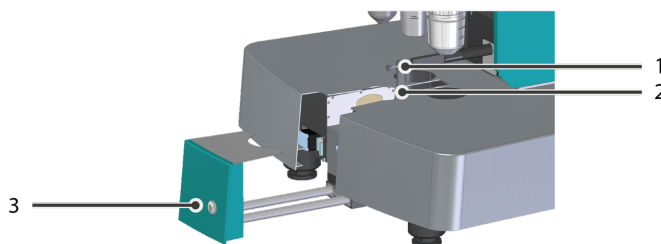
As long as the objectives are not in measurement position, do use a laser power of maximal 5%.



- 1 Check if the instrument is switched on. See **Switching on the instrument on page 21**.
- 2 Click **Objective Lens Focus > Move Detection Unit Up** in the **Settings 1** pane. Wait until the detection unit is in its park position.

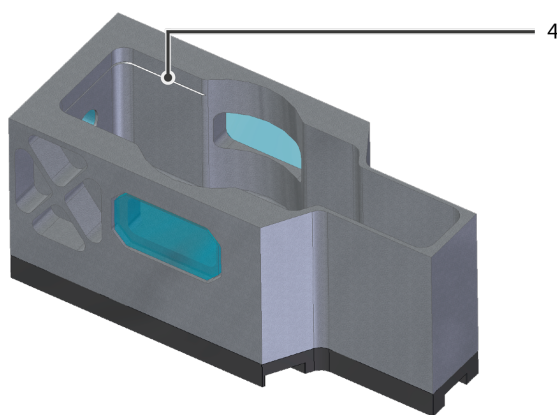


- 3 Pull out the drawer (3) as far as possible to lower the cuvette. The LED of the button on the drawer lights up solidly.



- 4 Dismount the sample arm (2) by loosening the thumb screw (1).
- 5 Remove the cuvette by lifting it from its magnetic holders.
- 6 Dispose of the imaging solution in accordance with local regulations.

- 7 Fill the cuvette up to the notch (4) on the side with the selected imaging solution.



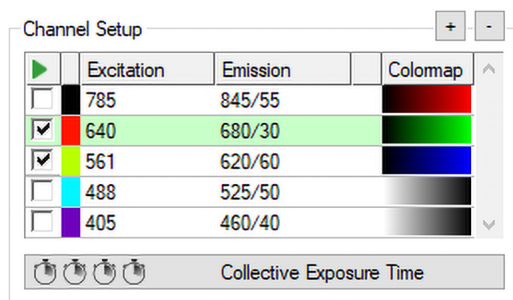
- 8 Place the cuvette securely on its magnetic holders again.

NOTICE Be careful not to spill any imaging solution. If hazardous or potentially infectious material has been spilled, decontaminate the affected area. Use an appropriate disinfectant.

- 9 Fasten the sample arm by tightening the thumb screw again.
- 10 Press the button on the drawer to lift the cuvette. The LED goes out.
- 11 Close the drawer.

6.2 Setting the parameters for the alignment

- 1 Select the appropriate **Instrument Mode** in the **Measurement Wizard**. See **Instrument Mode** on page 35.
- 2 Select the excitation and emission wavelengths under **Channel Setup** in the **Settings 1** pane of the **Blaze** view. For the alignment tool, the excitation wavelength is 561 nm. The selection must be highlighted to become active.



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



- 4 Set **Light Sheet Properties > Sheet NA** to the maximum.



- 5 Set **Light Sheet Properties > Sheet Width** to 100%.

- 6 Under **Optical Setup**, select the 4x objective lens (**LVBT 4x**) with 1.0x zoom, and select the imaging solution used in the cuvette for **Liquid**.

Optical Setup

Objective	Zoom	Liquid
LVBT 4x ▾	1.0x ▾	ECI ▾

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

7

Light sheet 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 at the bottom of the instrument can be used to align the individual beams creating the light sheet. 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. Screws z2 and z4 are used for aligning the outer light sheets.

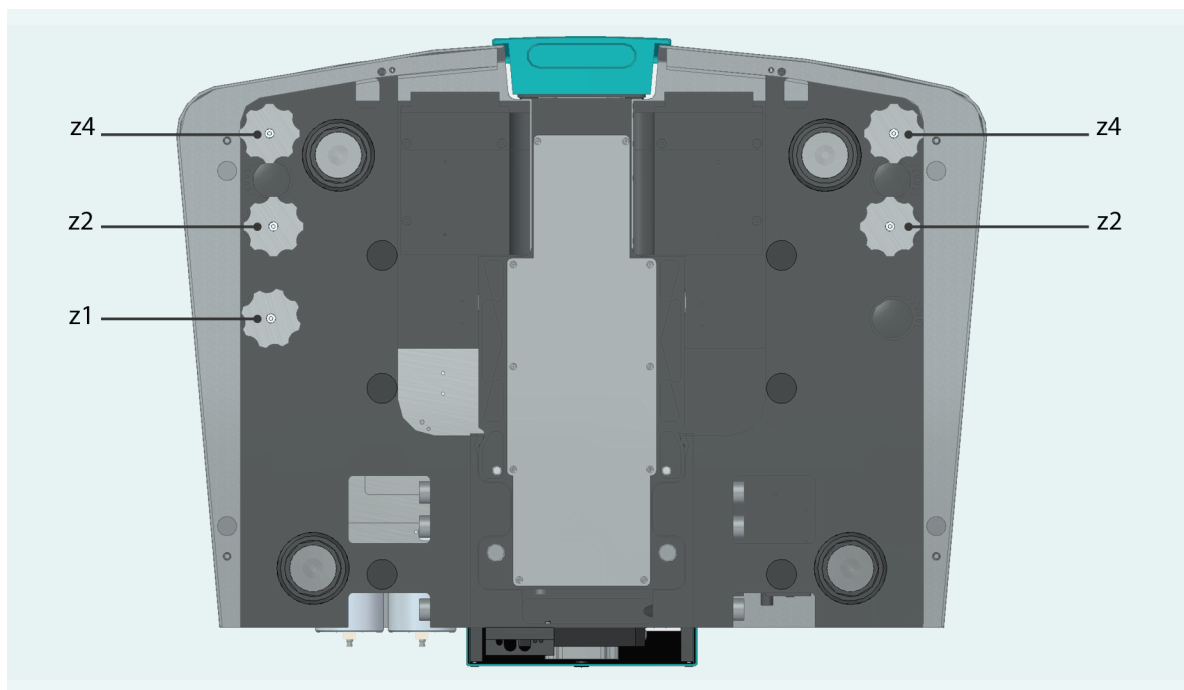


Figure 7.1: Bottom view of the instrument with the positions of the adjustable precision screws

7.1 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.

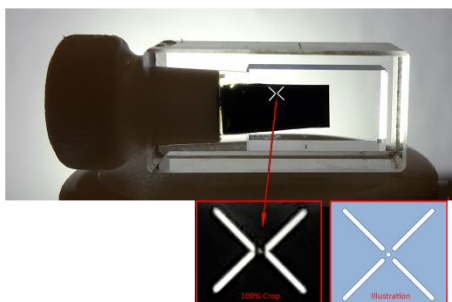
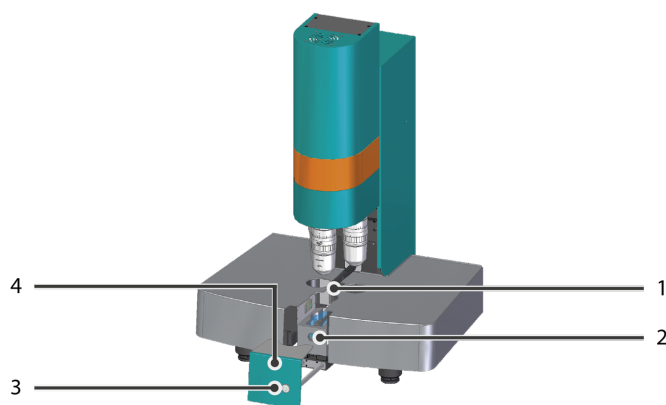


Figure 7.2: Side view of the alignment tool

7.2 Placing the alignment tool

- 1 Check if the instrument is switched on. See **Switching on the instrument on page 21**.
- 2 Pull out the drawer (4) as far as possible to lower the cuvette (2).



- 3 Lift up the sample arm (1) and place the alignment tool.
- 4 Lower the sample arm and fasten it in a centered position.
- 5 Press the button (3) on the drawer to lift the cuvette.
- 6 Close the drawer.

7.3 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.

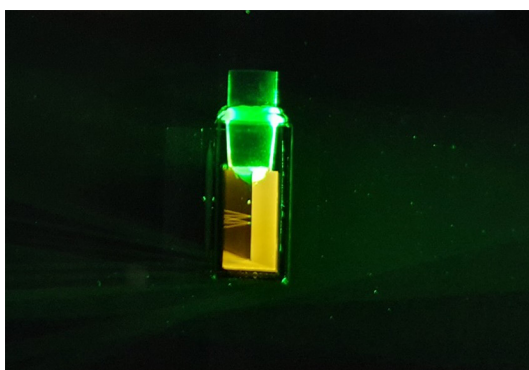
- 1 Click **Light Sheet Selection > Select right light sheet** in the **Settings 1** pane.



- 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 must pass the pinhole and form thin lines on the other side.



- 4 Click and hold the **Objective Lens Focus > Move Detection Unit Down** button to move down the objective lens. The detection unit automatically stops.

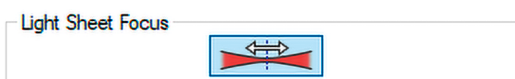


- 5 Focus on the light sheet by clicking the **Single Channel Autofocus** button. If it is difficult to focus properly in the autofocus mode, use the manual focus.

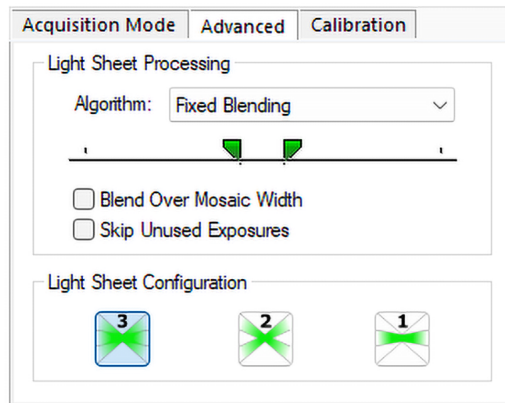


- 6 Click the **Show Crosshair** button on the right in the stack window to activate the crosshair, and line up the alignment tool in the center.

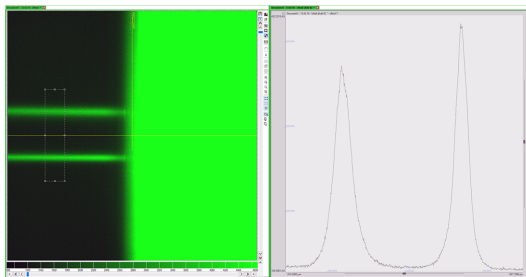
- 7 Activate the **Light Sheet Focus**, and place the light sheet focus indicator in the center of the field of view.



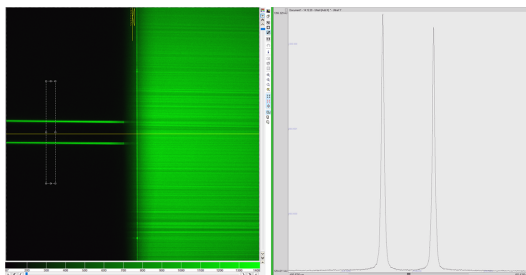
- 8 Select the **Advanced** tab in the **Settings 1** pane. Always start with the central light sheet on the right-hand side. It is the reference light sheet that cannot be adjusted by the user.



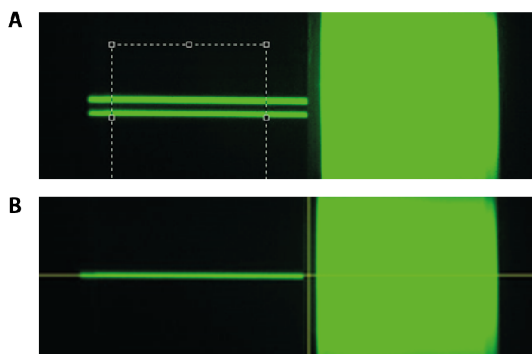
- 9 Click **Light Sheet Configuration > Enable only the central beam** to activate the central laser beam.
- 10 Draw a rectangle over the lines of the light sheet as the ROI, and adjust the contrast. If there are not any lines visible, move the **Light Sheet Focus Calibration** slider until the lines have a maximum sharpness. See **Adjusting the horizontal focus on the facing page**.



- 11 Right-click the ROI, and select **Add X** to add an intensity profile.
- 12 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.
- 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.



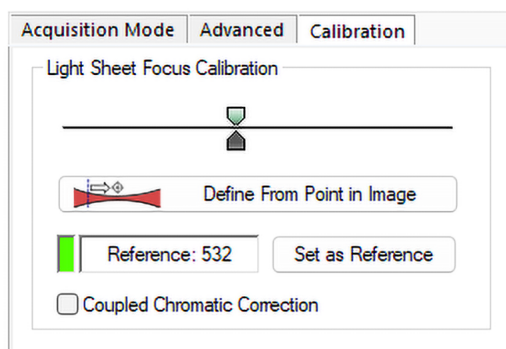
- 14** If two lines are visible passing the mask (**A**), lower or raise the alignment tool by using the mouse wheel. If the lines move closer to each other, the mouse wheel is moved in the correct direction. The aim is to align the pinhole located in the center of the mask to the central light sheet. 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 **Calibration** tab on the bottom of the **Settings 1** pane.
- 2 Move the top marker of the **Light Sheet Focus Calibration** slider until only one line passes the pinhole. Ensure that the light sheet focus is always in the center and the **Light Sheet Properties > Sheet NA** is at the maximum. If necessary, readjust the z-direction.



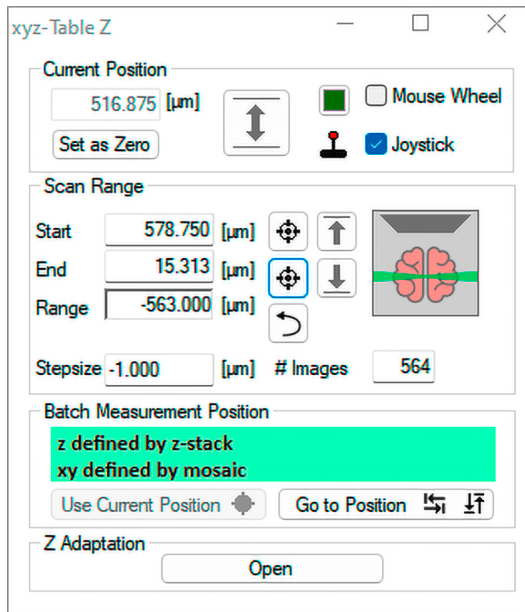
- 3 If only one line passes the pinhole in the center of the mask, activate the intensity profile.
- 4 Increase the intensity of this light sheet by switching between moving the alignment tool in z-direction and moving the slider. If not any further increase of the intensity is measurable, the pinhole is aligned to the light sheet.



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



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



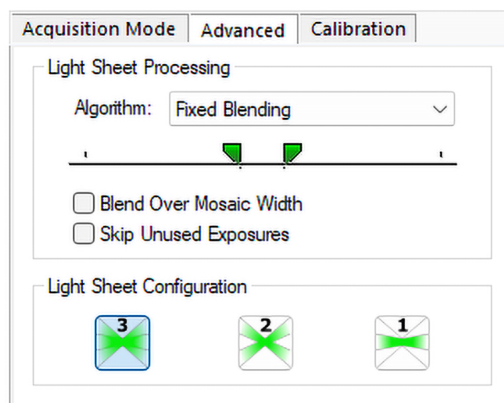
After the settings have been saved, the alignment tool must not be moved again. It is highly recommended to block the third button from the left of the jog wheel.

7.4 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.



- Click **Light Sheet Configuration > Enable all three beams** in the **Advanced** tab to activate all three laser beams.



- Adjust the height of the lateral light sheets. Start with the rear light sheet by using the screw z2 below the system. See **Figure 7.1**.
- Repeat the procedure with the front light sheet by using the screw z4.



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



- Switch between **Fit best** and **Grow** to rescale the graph.
- 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 current position** button.
Alternatively, use the mouse wheel.

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

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

- 1 Click **Light Sheet Selection > Select left light sheet** in the **Settings 1** pane.



- 2 Click **Light Sheet Configuration > Enable only the central beam** in the **Settings > Advanced** tab to activate the central laser beam.
- 3 Ensure that the slider for the horizontal focus is still in the center and the **Light Sheet Properties > Sheet NA** is at maximum. If necessary, adjust the position.
- 4 Adjust the height by using the screw z1. See **Figure 7.1**. If it is not possible to make only one light sheet pass the pinhole, see **Adjusting the horizontal focus on page 31**.

7.6 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 **Light Sheet Configuration > Enable all three beams** in the **Advanced** tab 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 below the instrument. See **Figure 7.1**.
- 3 Repeat the procedure with the front light sheet by using the screw z4.
- 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** in the **Current Position** group box.



- 8 Press the **Move to current 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.

8

Setting up an experiment

8.1 Placing the sample

- 1 Attach the sample to the sample holder.
- 2 Place the sample holder into the cuvette.

NOTICE Ensure that no medium gets into the microscope chamber.

8.2 Instrument Mode

In the **Measurement Wizard**, you can select the **Instrument Mode** for the ordered variant.

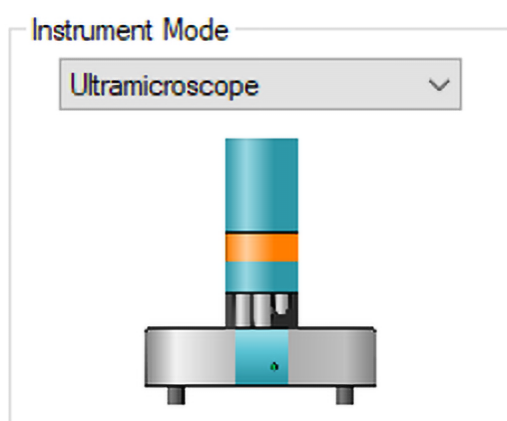


Figure 8.1: Instrument Mode list

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 55**.

8.3 Measurement Mode

To acquire data, you must define the order of the devices to be used during acquisition. The user can freely define these devices by selecting them in the **Devices** lists or use predefined settings, called measurement modes. The available measurement modes can be selected in the **Measurement Mode** list.

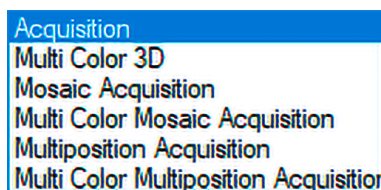


Figure 8.2: Measurement Mode list

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 55**.

8.3.1 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 **Optical Setup > Objective**, select the appropriate objective lens, the **Zoom** and the imaging solution used in the cuvette for **Liquid** in the **Settings 1** pane.

Optical Setup

Objective	Zoom	Liquid
LVBT 4x	1.0x	ECI

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

Channel Setup

	Excitation	Emission	Colormap
☐	785	845/55	
☒	640	680/30	
☒	561	620/60	
☐	488	525/50	
☐	405	460/40	

Collective Exposure Time

- 3 To set the current excitation wavelength as the new reference wavelength, click the **Set as Reference** button in the **Calibration** tab.

Acquisition Mode | Advanced | Calibration

Light Sheet Focus Calibration

☐ Coupled Chromatic Correction

- 4 Move the slider under **Laser Transmission Control** to set the laser power.
NOTICE Higher laser power bleaches the sample faster.
- 5 Click **Light Sheet Selection > Select right light sheet**.

Light Sheet Selection



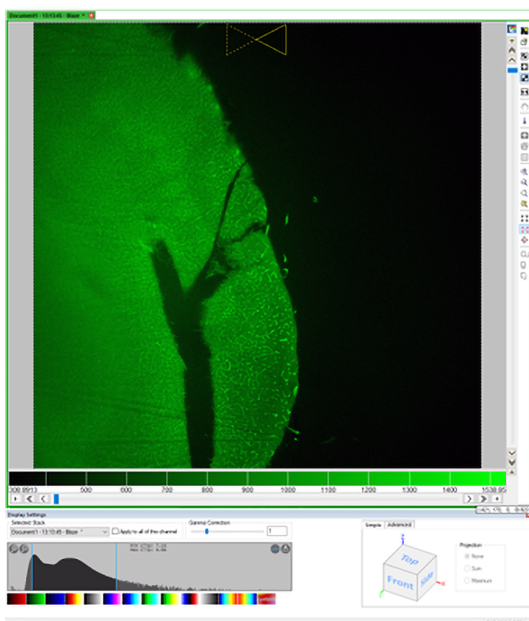
- 6 Click the **Start Live Preview** button in the **Measurement Wizard** to start the live preview.



- 7 Click and hold the **Objective Lens Focus > Move Detection Unit Down** button to move down the objective lens. The detection unit automatically stops.



- 8 Use the jog wheel to move the sample inside the cuvette until the surface of the sample is visible in the FOV.
- 9 Focus on the sample.
- 10 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 top marker (**Global Offset**) of the **Light Sheet Focus Calibration** slider. Alternatively, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



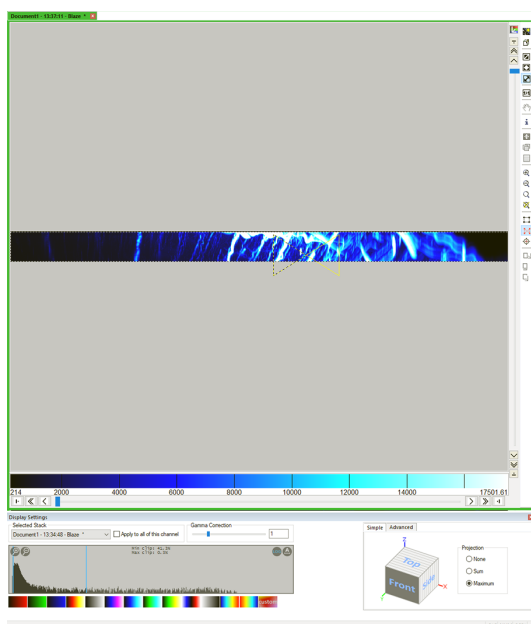
- 11 Move the light sheet focus indicator along the sample to check if the contrast is always best below.
- 12 Set up a z-stack of about 200 micrometers with two micrometers step size to confirm the calibration.



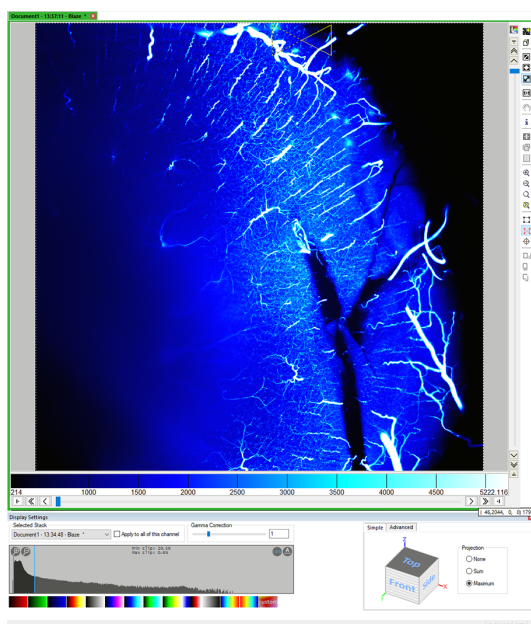
- 13 Select **Measurement Mode > Acquisition** and deactivate autosave.



- 14 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, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



- 15 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, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



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



- 17 Click **Save Settings** on the toolbar.

8.3.2 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 **Calibration** tab. These chromatic offsets in the excitation path have to be corrected using a sample.

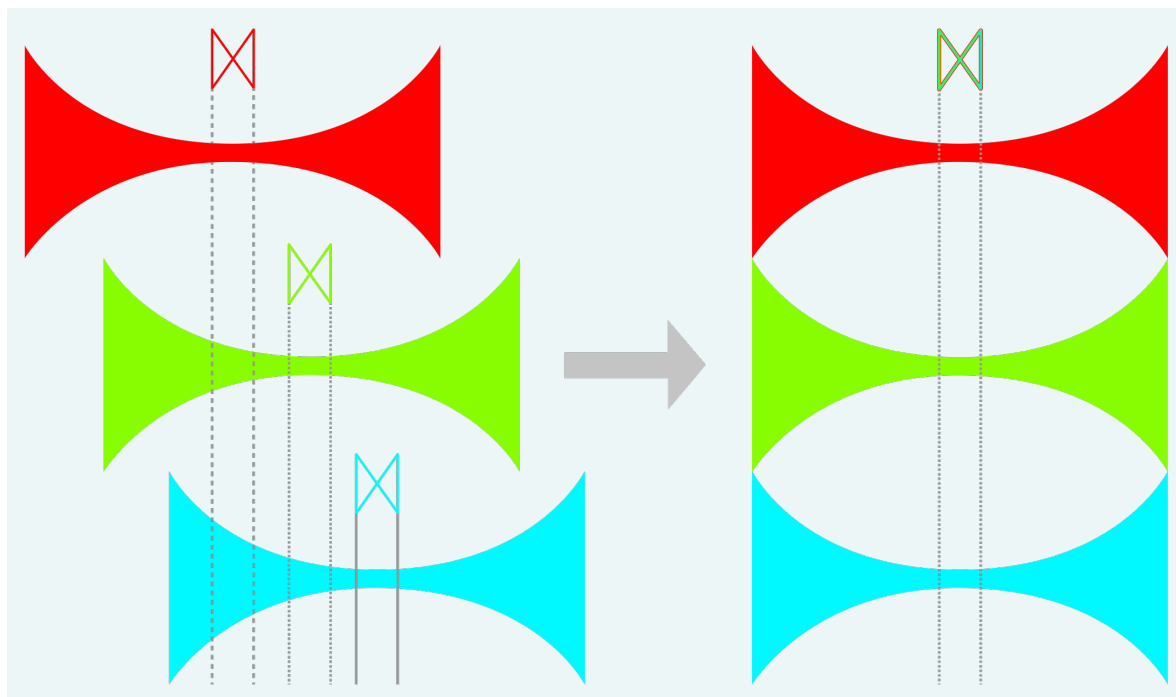
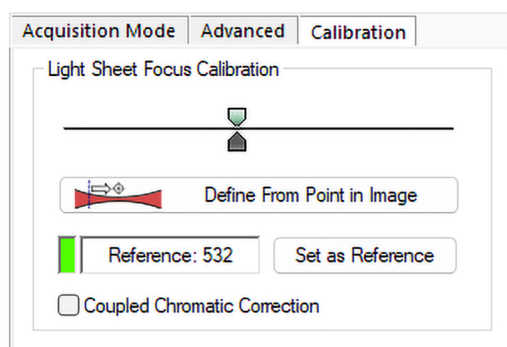


Figure 8.3: Chromatic offsets



- 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 bottom marker (**Chromatic Correction**) of the **Light Sheet Focus Calibration**. The marker changes color to the set color under **Channel Setup**.
Alternatively, click **Define From Point in Image** and place the crosshair by clicking on the actual position of the light sheet focus.



- 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 36**. If necessary, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.
- 6 If **Coupled Chromatic Correction** is selected, the chromatic correction is applied symmetrically to the left and right light sheet. Otherwise, the chromatic correction has to be set individually for both light sheets.
- 7 Click **Save Settings** on the toolbar.



8.3.3 Acquiring an image



- 1 To start the live preview, click the **Start Live Preview** button in the **Measurement Wizard**.
- 2 Move in the z-direction to the start position.
- 3 Click **Current Position > Set as Zero** in the **Settings 2** pane.



4 Click the **Set current position as start** button under **Scan Range > Start**.

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



6 Click the **Set current position as end** button under **Scan Range > End**.

7 Define the step size, corresponding to the number of images, by entering it under **Scan Range > Stepsize**.



8 To stop the live preview, click the **Stop Live Preview** button. The laser is switched off.

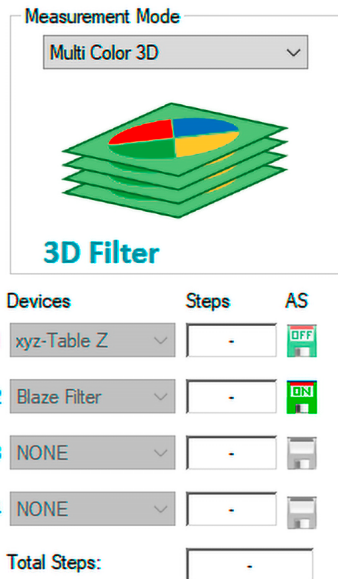
9 Check **Settings** in the **Autosave** area of the **Experiment Manager**.



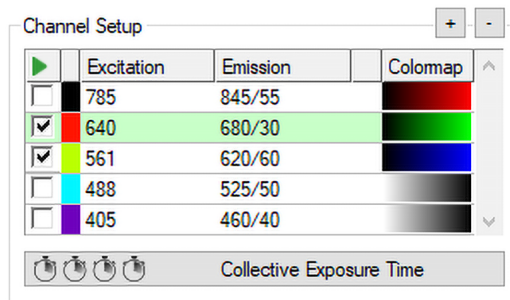
10 To start the acquisition, click the **Start Measurement** button.

8.3.4 Acquiring a multicolor z-stack

- 1 In the **Measurement Wizard**, select **Measurement Mode > Multi Color 3D**.



- 2 In the **Devices** list, select **xyz-Table Z** and **Blaze Filter**.
- 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** 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**, starting with the next longer wavelength.



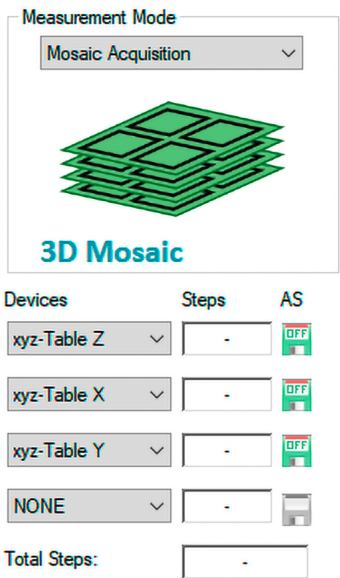
- 8 If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.



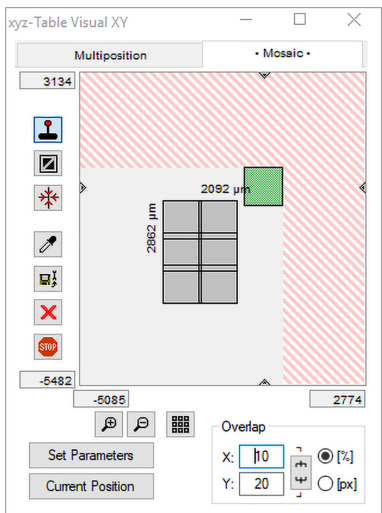
- 9 To start the acquisition, proceed as described under **Acquiring an image on page 39**.

8.3.5 Acquiring a 3D mosaic image

- 1 In the **Measurement Wizard**, select **Measurement Mode > 3D Mosaic**.



- 2 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.

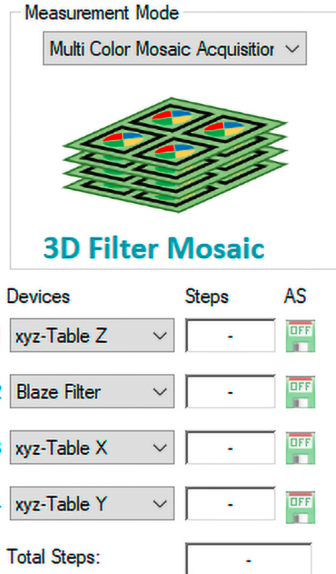


- 3 Delete all positions of this **Measurement Mode**.
- 4 Double-click the green tile representing the field of view. The tile turns gray.
- 5 Click and drag the black dots at the edges to create a mosaic.
- 6 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).
- 7 Click the **Autosave** button at the end of the row under **AS**. The button turns green.
- 8 To start the acquisition, proceed as described under **Acquiring an image on page 39**.



8.3.6 Acquiring a multicolor mosaic image

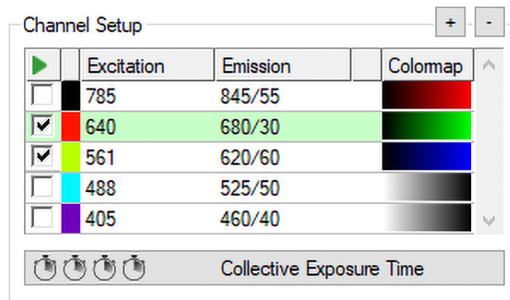
- 1 In the **Measurement Wizard**, select **Measurement Mode > Multi Color Mosaic Acquisition**.



- 2 In the **Devices** list, select **xyz-Table Z** and **Blaze Filter**.



- 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** 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.



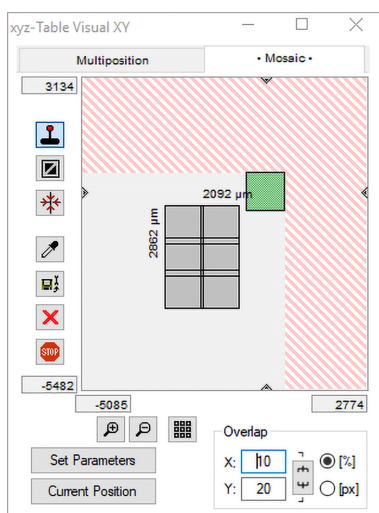
- 7 Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction**, starting with the next longer wavelength.



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



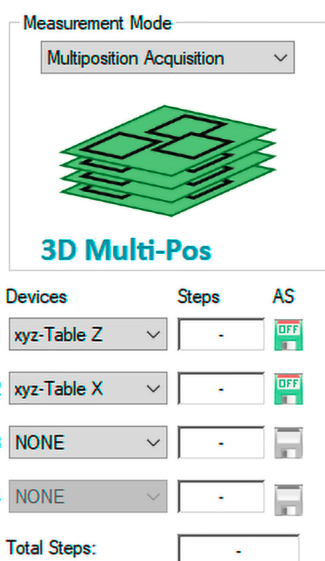
- 9 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.



- 10 Double-click the green tile representing the field of view. The tile turns gray.
- 11 Click and drag the black dots at the edges to create a mosaic.
- 12 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).
- 13 To start the acquisition, proceed as described under **Acquiring an image on page 39**.

8.3.7 Acquiring a 3D multiposition dataset

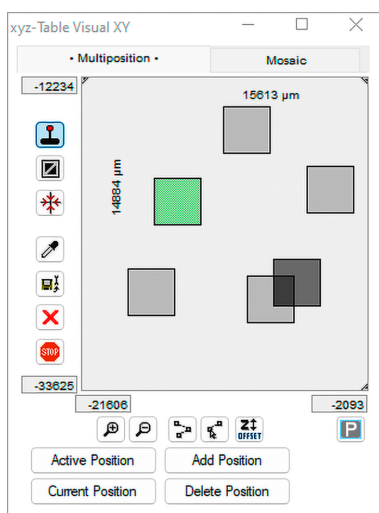
- 1 In the **Measurement Wizard**, select **Measurement Mode > Multiposition Acquisition**.



- 2 In the **xyz-Table Visual XY** view, click the **Multiposition** tab.
- 3 Delete all positions of this measurement mode.
- 4 Move the measurement positions to your region of interest.



- 5 Click **Add Position** for each position you want to add.



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

- 7 To start the acquisition, proceed as described under **Acquiring an image on page 39**.

8.4 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.



2 Click the **Auto Contrast** 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 Select a suitable acquisition mode:

- **Dynamic Focus**
- **Fast Tiling Scan**
- **LightSpeed Mode** (only available if ordered)

5 Optional: Move the slider under **Light Sheet Properties > 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.

6 Optional: Move the slider under **Light Sheet Properties > Sheet Width** to reduce 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.

9

Maintenance

9.1 Servicing

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

9.2 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.

9.3 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.

10

Technical data and specifications

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

UltraMicroscope Blaze Imaging System

General information

Dimensions (w × d × h)	670 mm × 525 mm × 910 mm
Weight	96 kg (without laser module)
Nominal input voltage	24 V DC, provided by external power adapter
Fuse	4 A time lag

Working conditions

Temperature	15–35 °C
Humidity	Max. 70% relative humidity, noncondensing
Altitude	Max. 2,000 m
Emission sound pressure level	<48 dB(A)

Ambient conditions

Overvoltage category	II
Pollution degree	2

Storage conditions

Temperature	4–55 °C
Humidity	Max. 70% relative humidity, noncondensing

Transport conditions

Temperature	4–55 °C
Humidity	Max. 70% relative humidity, noncondensing

Table 10.1: Technical data and specifications of the instrument

The instrument may have one of the following configurations. The specifications from the **Basic** variant also apply to the other variants with the exception of the laser module.

Basic**Cuvette**

Imaging solutions	Aqueous buffers or organic solvents
Sample travel range (x, y, z)	24 mm, 50 mm, 23 mm
Cuvette size (inside dimensions w × d × h)	51 mm × 129 mm × 64 mm
Refraction index matching	1.33–1.56

Laser module

BC	3 laser lines (488, 561, and 639 nm), 75–150 mW per diode
Laser class	3B

Detection optics / objective lenses 1.1x, 4x, and 12x

Emission filters (Ø 43 mm)	3 filters
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

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 10.2: Technical data and specifications of the **Basic** variant

Advanced

Laser module

BC	4 laser lines (488, 561, 639, and 785 nm), 75–150 mW per diode
Laser class	3B

Detection optics / objective lenses 1.1x, 4x, and 12x

Emission filters (Ø 43 mm)	4 filters
Chromatic correction	Dynamic 400–850, nm

Table 10.3: Technical data and specifications of the **Advanced** variant

Fully featured

Laser module

BC	5 laser lines (405, 488, 561, 639, and 785 nm), 75–150 mW per diode
Laser class	3B

Detection optics / objective lenses 1.1x, 4x, and 12x

Emission filters (Ø 43 mm)	5 filters
Chromatic correction	Dynamic 400–850, nm

Table 10.4: Technical data and specifications of the **Fully featured** variant

White Light Laser

Laser module

Supercontinuum white light laser	405–850 nm spectral range, 1–4 mW/nm
Laser class	4

Table 10.5: Technical data and specifications of the **White Light Laser** variant

10.1 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 Blaze 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 Brempe
Miltenyi Biotec B.V. & Co. KG
Astastraße 14
33617 Bielefeld
Germany

10.2 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 Blaze 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 Brempe
Miltenyi Biotec B.V. & Co. KG
Astastraße 14
33617 Bielefeld
Germany

11

Technical support

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

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone: +49 2204 8306 3803
technicalsupport@miltenyi.com

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

12

Legal notes

12.1 Limited warranty

Except as stated in a specific warranty statement which may accompany this product or as otherwise agreed in writing by an authorized representative of Miltenyi Biotec, Miltenyi Biotec's warranty to you, the original purchaser and end user ("you" or "your"), with respect to the product accompanied by this limited warranty shall be subject to the following provisions and the general terms and conditions of sale of the company within the Miltenyi Biotec group which supplied the product in effect at the date of purchase. Those terms and conditions of sale may vary by country and region. Nothing in this document should be construed as constituting an additional warranty.

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