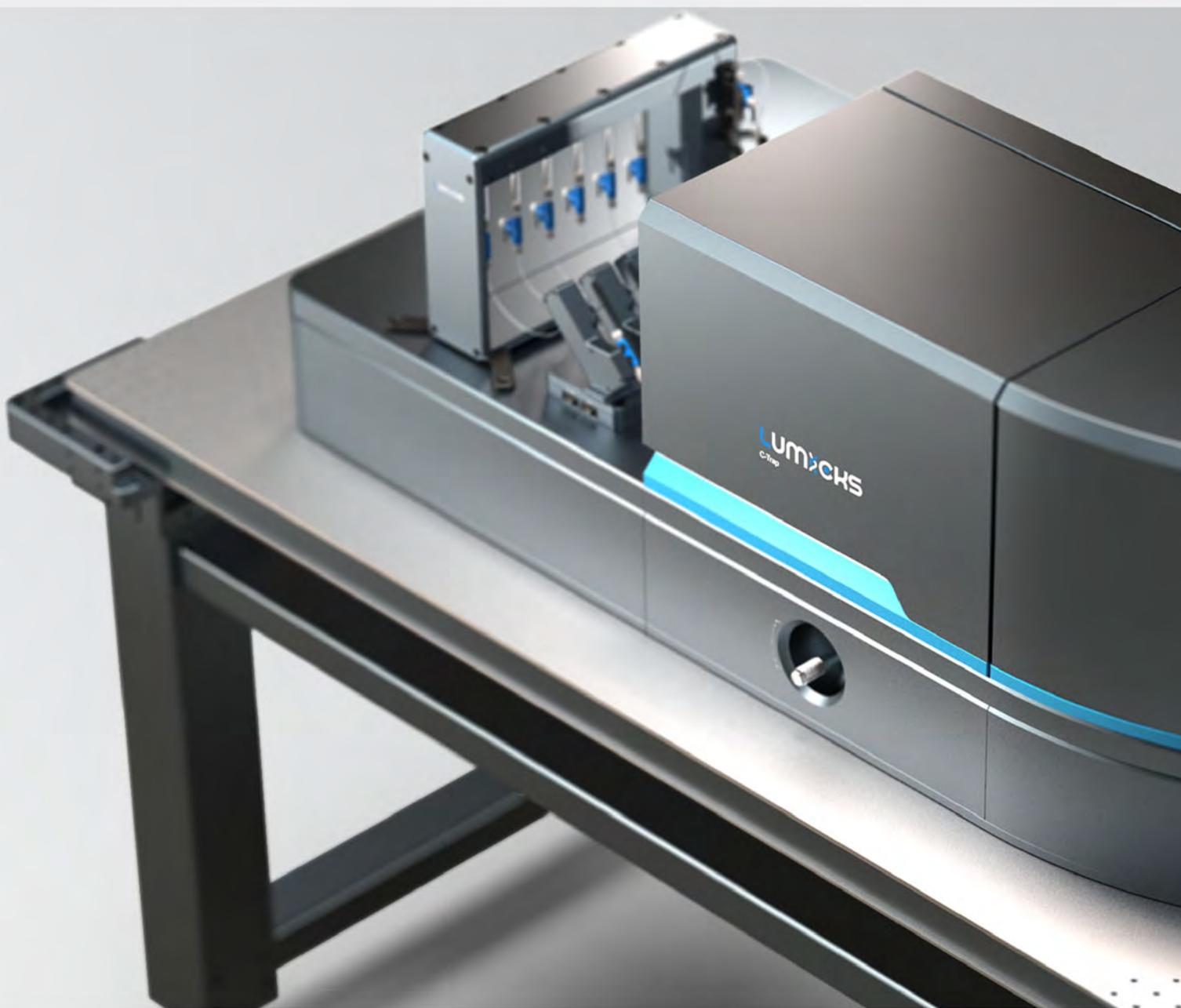


C-Trap® Optical Tweezers-Fluorescence Microscope

User Manual

For research use only – Version 4



LUMICKS

Contents

1	Disclaimer	1
2	Revision history	3
3	CE Declaration of Conformity	7
4	General description of C-Trap	9
5	About this user manual	11
6	Symbols used in this user manual	13
7	General safety, maintenance and service	15
8	Laser safety	19
8.1	Warning and safety labels	19
9	Cautions and notes	23
10	System overview	25
10.1	Overview of the C-Trap instrument	25
10.2	Electronics rack	26
10.3	Workstation	26
11	Basic operations	29
11.1	Starting up of the system and software	29
11.2	Homing the sample stage	31
11.3	Adjusting the microscope objective correction collar	32
11.4	Adding immersion liquids for the microscope objective and condenser	33
11.5	Turning on the trapping laser	35
11.6	Finding the correct z-position of the microscope objective	37
11.7	Finding the correct z-position of the condenser	39
11.8	Flowcell selection & visualization	42
11.9	Force calibration	43
11.10	Fluorescence excitation lasers and confocal scan settings (C-Trap Dymo)	45
11.11	Super-resolved fluorescence imaging (C-Trap Dymo 700)	45
11.12	Fluorescence excitation lasers and Widefield/TIRF camera settings (C-Trap Edge)	47
11.13	IRM LED and camera settings (C-Trap Edge)	52
11.14	Focus lock (C-Trap Edge)	52
11.15	Q-trap settings (C-Trap Dymo 400, Dymo 700 and Edge 450)	54
11.16	Temperature Control	55
11.17	Time line channels	56
11.18	Shutting down the system (end of day)	58

11.19 Shutting down the system for a short break	59
11.20 Waste disposal	60
12 Appendix	61
12.1 U-Flux - Automatic fluidics setup	61
12.2 Exchanging the flowcell tubing	67
12.3 Aligning a sample using the tip-tilt sample holder	68
12.4 Bridge PC and Controller PC	70

1 Disclaimer

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2 Revision history

LUMICKS reserves the right to revise this document and/or to further develop the products described in this document at any time without prior notice or any other obligation. Information and specifications in this user manual are subject to change without notice.

Document title: C-Trap® User Manual

Document revision: 4.0

Date revision: Jul 24, 2024

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Printed in The Netherlands.

Table 2.1: Revision history of the C-Trap® User Manual.

Revision	Description of revision	Date
1	<ul style="list-style-type: none"> Initial version. 	11.11.2015
2	<ul style="list-style-type: none"> Added automated condenser to the Quick guide section. Updated software. 	29.01.2016
2.1	<ul style="list-style-type: none"> Restructured Quick guide section. Modified section “Finding the correct position of the objective”. Addition to appendix. 	19.02.2016
2.2	<ul style="list-style-type: none"> Modified appendix (specific data for laser powers). 	25.02.2016

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Table 2.1 – continued from previous page

Revision	Description of revision	Date
2.3	<ul style="list-style-type: none"> • Restructured section 9.3. • Added new sections 9.9, 10.3, 10.6, 10.7, 10.8, 10.9. • Moved laser power settings from appendix to a separate document. 	24.03.2016
2.3.1	<ul style="list-style-type: none"> • Modified section “Fluorescence laser and Scanary settings”. 	07.04.2016
2.3.2	<ul style="list-style-type: none"> • Modified section “Handling, cleaning and storage of glass flow cell”. 	12.04.2016
2.3.3	<ul style="list-style-type: none"> • Added new section “Data storage”. • Added new section “Adjust objective correction collar”. • Added new section “Data analysis”. • Changing the section “Fluorescence laser and Scanary settings”. • Spelling corrections. 	28.07.2016
2.3.4	<ul style="list-style-type: none"> • Modifiedthe section for the manual condenser option. 	13.09.2016
2.4	<ul style="list-style-type: none"> • Updated the document for two condenser versions: manual and automated. 	23.09.2016
2.4.1	<ul style="list-style-type: none"> • Updated company address. • Updated pictures. 	28.10.2016
2.4.2	<ul style="list-style-type: none"> • Modified the document for non-confocal options. 	13.03.3017
2.4.3	<ul style="list-style-type: none"> • General refactor of the document. 	26.04.2017
2.5	<ul style="list-style-type: none"> • Modified for the new prototype software (special). 	24.10.2017
2.6	<ul style="list-style-type: none"> • Updated for CE declaration of conformity. 	28.02.2018
2.7	<ul style="list-style-type: none"> • Updated to add Bluelake software. 	17.05.2018

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Table 2.1 – continued from previous page

Revision	Description of revision	Date
2.7.1	<ul style="list-style-type: none"> Updated to add Temperature Control option. 	29.11.2018
2.7.2	<ul style="list-style-type: none"> Updated the name to C-Trap G2. 	23.01.2019
2.8	<ul style="list-style-type: none"> Added Widefield/TIRF & IRM imaging. Added focus lock feature. 	09.08.2019
2.9	<ul style="list-style-type: none"> Added instructions for the objective exchange (section 11.3). 	14.01.2020
3	<ul style="list-style-type: none"> General update of the user manual. Updated the objective exchange procedure. Updated Widefield/TIRF & IRM imaging section. 	06.04.2021
3.1	<ul style="list-style-type: none"> General update of the user manual. Updated the objective exchange procedure. 	16.06.2021
3.2	<ul style="list-style-type: none"> General update of the user manual. Moved objective exchange procedure to a separate set of instructions. Moved the alignment procedure of the condenser to a separate set of instructions. Updated Bluelake screenshots to version 2.1. Changed maximum power of STED depletion laser and confocal lasers according to SAT. 	22.07.2021
3.2.1	<ul style="list-style-type: none"> Replaced C-Trap screenshots with the current version. Removed interlock defeat from screenshots. Minor changes in section 9. 	06.09.2022
3.3	<ul style="list-style-type: none"> Changes to section 13.1, updated procedure without interlock override. Correct usage of warnings and notices. Product names changed to new configuration families: C-Trap, C-Trap Dymo and C-Trap Edge. Reformulated most instructions and corrected errors. 	20.07.2023

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Table 2.1 – continued from previous page

Revision	Description of revision	Date
4.0	<ul style="list-style-type: none">Added new section in the appendix on the fluidics setup.Convert format from Word document to restructured text.	09.01.2024

3 CE Declaration of Conformity

Here you can find the CE declaration of conformity, see [Figure 3.1](#).

EC DECLARATION OF CONFORMITY

This Declaration of Conformity is issued under the sole responsibility of the manufacturer

Manufacturer

Company name:	LUMICKS B.V.
Full address:	Paalbergweg 3
Postal code:	1105 AG
Place:	Amsterdam
Country:	The Netherlands
Person authorized to compile the Technical File:	Siegfried Weisenburger

Description and Identification of the machinery

Generic name:	C-Trap
Function:	Simultaneous manipulation and visualization of single molecule interactions in real time
Model:	C-Trap G2
Type:	09, large and extra large
Serial number:	00001-99999
Commercial name:	C-Trap TM

Compliance

The manufacturer declares that the above mentioned machinery fulfills all relevant provisions of

Machinery Directive 2006/42/EG
Low Voltage Directive 2014/35/EU
EMC Directive 2014/30/EU
RoHS Directive 2011/65/EU

In conjunction with the following harmonised standards and where appropriate other technical standards and specifications

EN ISO 12100:2010; EN 349:1993+A1:2008; EN 61010-1:2010; EN 61326-1:2006
EN 60825-1:2014; EN 55011:2009; EN 61000-4-2:2009; EN 61000-4-5:2014;
EN 61000-4-3:2006; EN 61000-4-6:2014; EN IEC 61000-4-11:2004

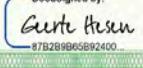
Place:	Amsterdam The Netherlands	Name: Geerte Heesen Function: Chief of Staff
Date:	August 1, 2023	Signature:  97B269965B92400...

Figure 3.1: CE Declaration of Conformity.

4 General description of C-Trap

The LUMICKS C-Trap is a Correlative Tweezers-Fluorescence Microscope (CTFM) instrument. It integrates high-resolution optical tweezers with various imaging techniques such as confocal microscopy, STED (Stimulated Emission Depletion) super-resolved fluorescence microscopy, and Widefield/IRM (Interference Reflection Microscopy)/TIRF imaging. It also incorporates an advanced microfluidics system, providing the user with a comprehensive and correlated solution to manipulate and visualize molecular interactions in real-time.

The C-Trap allows to investigate the dynamic behavior of individual molecules, explore heterogeneity, and unravel mechanisms of action.

Firstly, optical tweezers enable the manipulation of biomolecules with sub-nanometer precision, and the exertion and measurement of forces with sub-piconewton resolution.

Secondly, fluorescence microscopy (Confocal/Widefield/TIRF) allows imaging individual molecules at the diffraction limit. As a result, biological processes can be monitored at the highest level of detail. In addition, IRM allows for highly sensitive label-free imaging at the flow cell surface.

Lastly, multi-channel laminar flow microfluidics allow the introduction of reagents and precious samples into the flow cell, in a controlled manner, and the in-situ assembly of a wide range of complex, multi-step single-molecule assays.

The C-Trap was specifically designed for optical trapping microspheres and tethering biomolecules, such as DNA, in between the trapped objects using established conjugation chemistry. The tethered biomolecules can then be manipulated with the high-resolution optical tweezers, while simultaneously measuring force, extensions, and fluorescence microscopy data.

5 About this user manual

This user manual primarily focuses on providing essential safety instructions that must be observed when working with the C-Trap. These instructions are crucial to ensure user safety and the proper operation of the equipment.

In addition to safety instructions, this manual also provides an overview of the operating principles of the C-Trap. Understanding these principles will help users familiarize themselves with the system's components and functionalities.

Furthermore, this manual presents a step-by-step guide on starting and preparing the C-Trap for experiments. By following these instructions, users can ensure that the system is properly set up and ready for conducting their experiments.

For any instructions not covered in this manual, users are advised to refer to the [LUMICKS help page](https://help.lumicks.com) (<https://help.lumicks.com>) or contact LUMICKS personnel for further assistance.

6 Symbols used in this user manual

In this user manual, the following symbols are used.

Warning: It indicates a hazardous situation which, if not avoided, could result in death or serious injury.

Note: It indicates a situation which, if not avoided, could result in product damage as well as property damage.

7 General safety, maintenance and service

The C-Trap is advanced scientific equipment and should only be operated by trained professionals. Although LUMICKS has taken measures to ensure easy and safe operation, users must receive professional training in general laboratory safety and be knowledgeable about electronic and laser equipment safety.

The C-Trap family instruments include high-power lasers, some of which emit invisible near-infrared (NIR) radiation. While LUMICKS has aimed to design a safe and user-friendly instrument, the user is responsible for their own safety. The system is intended for use within a laboratory setting only.

The optics in the microscope unit have been designed to prevent any harmful laser radiation emission during normal operation. The front lid of the sample area contains a safety interlock mechanism (refer to [Figure 7.1](#) and [Figure 7.2](#)) that automatically shuts off the high-power NIR laser when opened. Other compartments containing laser radiation are securely closed with screws.

Airborne noise emission levels are below 70 dB (A-weighted SPL) at all user accessible working areas near the instrument.

Warning: The user must never open the maintenance lids, and/or operate the instrument with open or removed maintenance lids.

The C-Trap instruments are not certified by any certifying body and are not a consumer product. LUMICKS cannot be held liable for any improper use, misuse or for use outside the specifications as well as any risks resulting from it.

Maintenance, repairs, modifications, removal or exchange of components, and any other interference with the equipment beyond the procedures outlined in this manual may only be performed by the LUMICKS personnel or under their direct guidance.

LUMICKS cannot be held liable for damage resulting from operating errors, negligence, or unauthorized alterations to the equipment, including the removal or replacement of instrument parts or the use of incompatible accessories from other manufacturers. Any such action will also void all warranty claims.

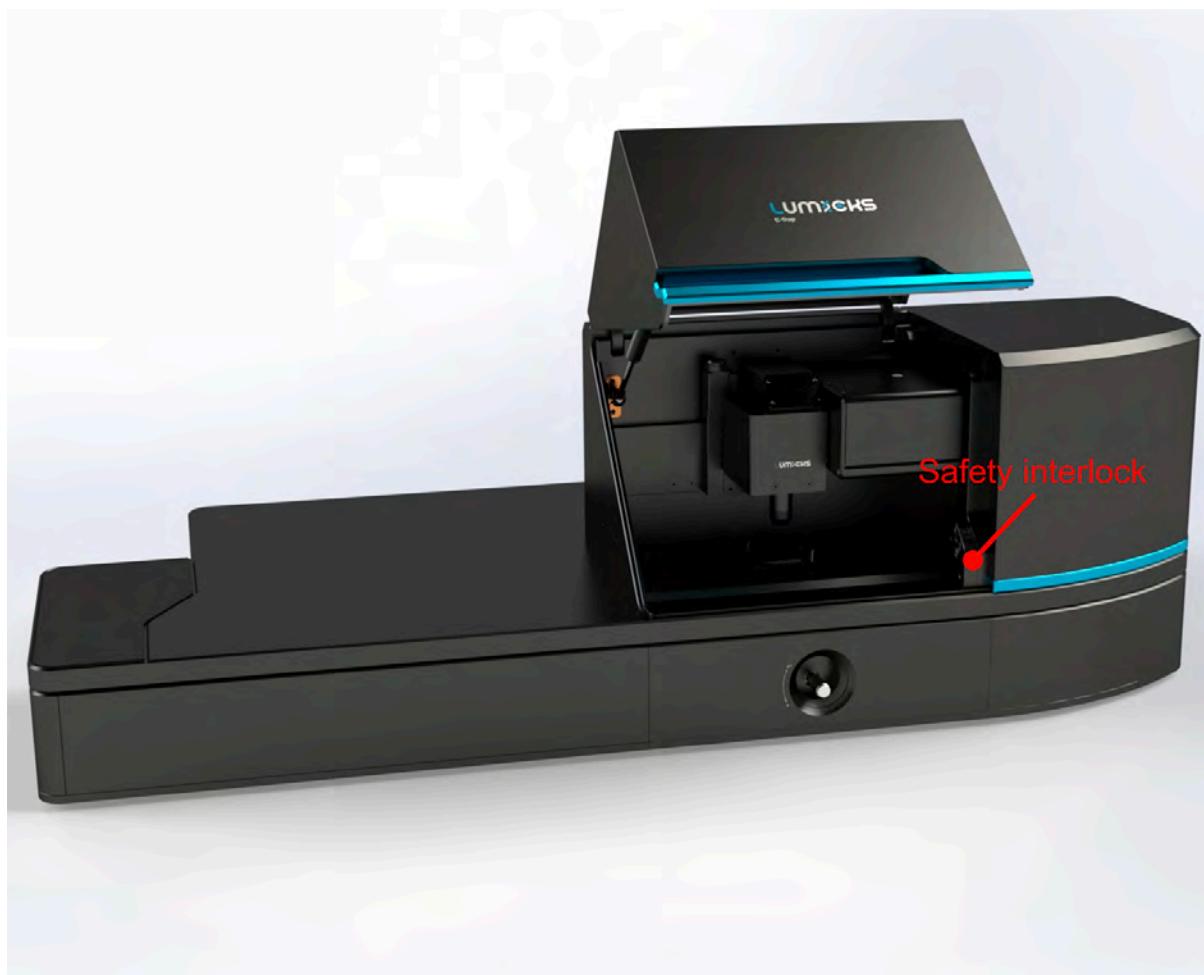


Figure 7.1: Location of the safety interlock on the C-Trap.



Figure 7.2: Front lid of the C-Trap providing access to the sample area.

8 Laser safety

The C-Trap family instruments are designed to operate safely under normal usage conditions and are classified as Laser Class 1. The instruments contain high-power lasers, including some that emit invisible near-infrared (NIR) radiation. Under normal operation, all laser emissions are fully contained within the device. The C-Trap family instruments also feature a safety interlock mechanism, which automatically turns off laser emission when the front lid to access the sample area is opened.

Warning: Do not attempt to defeat the safety interlock!

Warning: While the C-Trap instruments are classified as Laser Class 1 during regular use, they contain internal components that include Class 3B and Class 4 laser equipment. These lasers are exposed only during installation or service, and may only be accessed by trained personnel who are fully aware of the potential hazards. Failure to adhere to safety protocols will be at the operator's own risk.

8.1 Warning and safety labels

The warning and safety labels attached in the following locations to the C-Trap must be observed, see [Figure 8.1.1](#), [Figure 8.1.2](#), and [Figure 8.1.3](#).

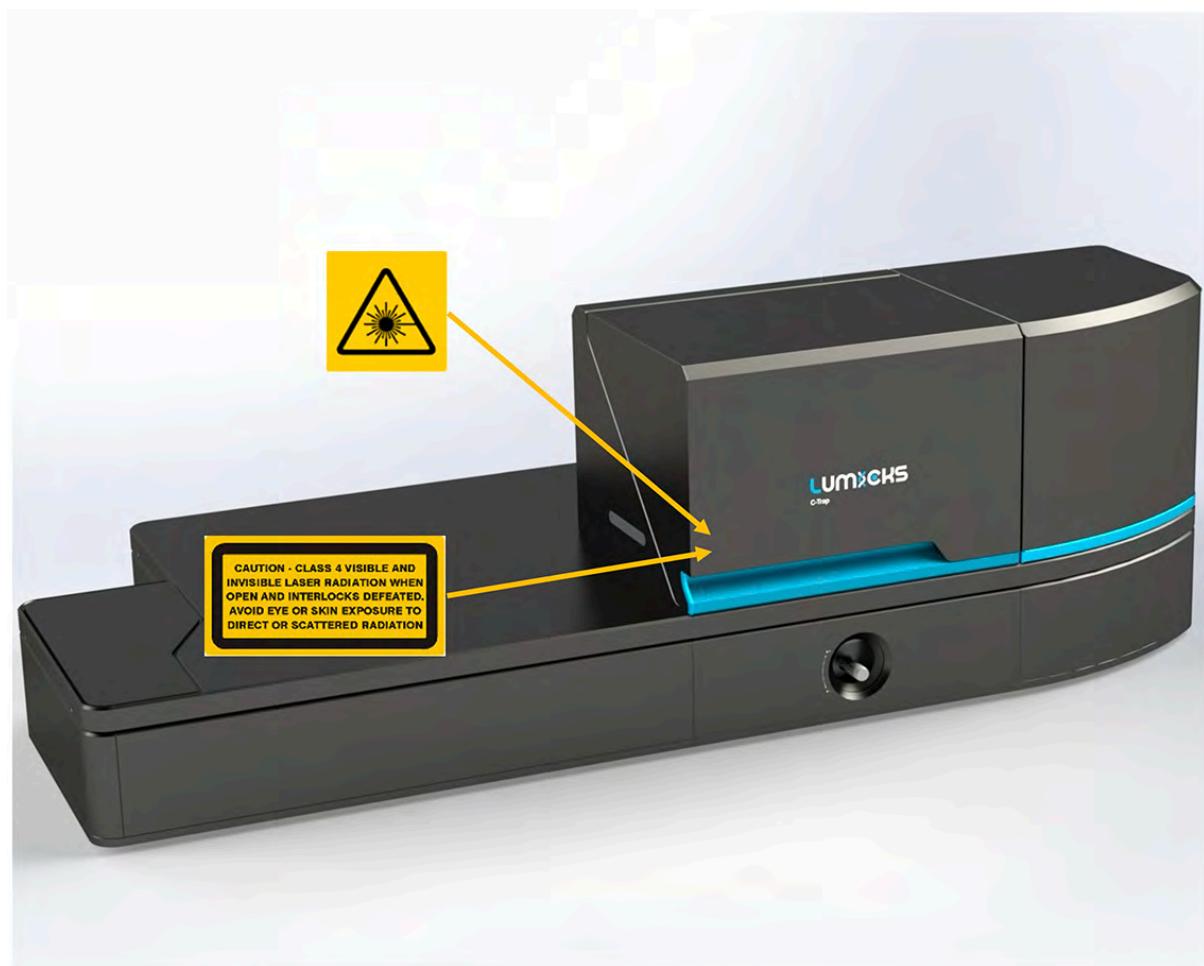


Figure 8.1.1: Warning and safety labels on the C-Trap (front view).



Figure 8.1.2: Warning and safety labels on the C-Trap (close-up open view).

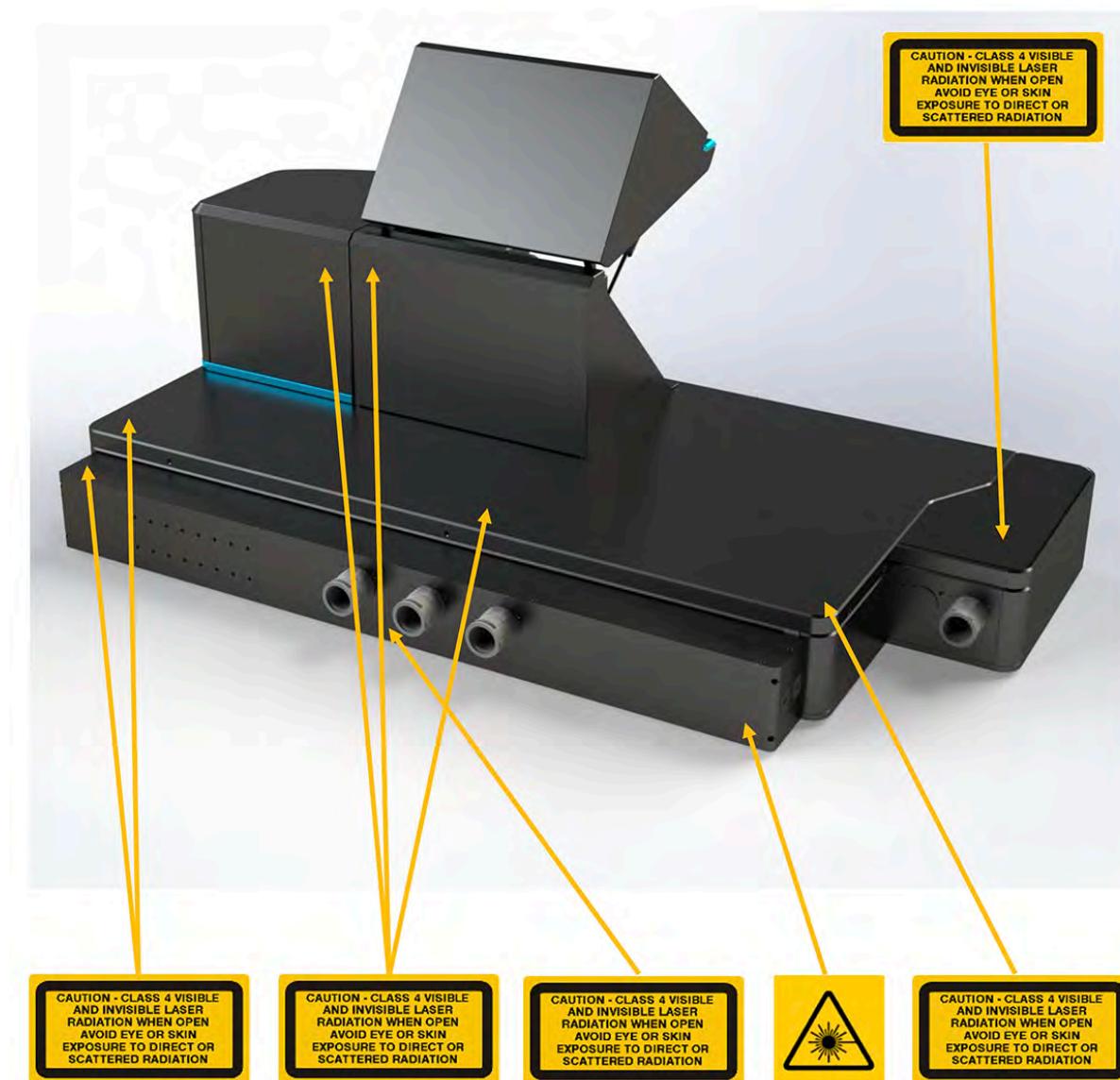


Figure 8.1.3: Warning and safety labels on the C-Trap (back view).

9 Cautions and notes

The C-Trap is a high-precision opto-electronic instrument. It is important to note that improper handling can potentially compromise its functionality or cause damage.

The user assumes full responsibility for operating the system correctly and safely, as well as ensuring its proper maintenance in accordance with applicable safety regulations. The user bears full liability for any consequences that may arise from using the system for purposes other than its intended design.

Note: Attention should be paid to the following notes.

Important notes:

- Avoid contact with liquids and prevent liquids from entering the system's housing.
- Do not expose the system to mechanical vibrations.
- Do not obstruct the unit's air ventilation slots and fan outlets.
- Do not subject the system to strong acoustic vibrations.
- Maintain a dust-free environment.
- Do not store any objects (such as tools or pipettes) on the system.
- Take special care when working with aggressive chemicals.
- Do not disconnect any cables from the system, PC, or the electronics rack unless explicitly instructed by LUMICKS personnel.
- Moving or relocating of the C-Trap instruments should only be done under the guidance of LUMICKS personnel.
- The installation and servicing of the system is always carried out by LUMICKS personnel. If you have any questions regarding servicing, please contact LUMICKS support via email (support@lumicks.com) or call +31 (0) 85 303 2745.
- For any other instruction not mentioned in this manual, please reach out to LUMICKS support.

Warning: In case of emergency or system breakdown pull the mains power-plug from the wall socket immediately and contact LUMICKS support at +31 (0) 85 303 2745.

10 System overview

10.1 Overview of the C-Trap instrument

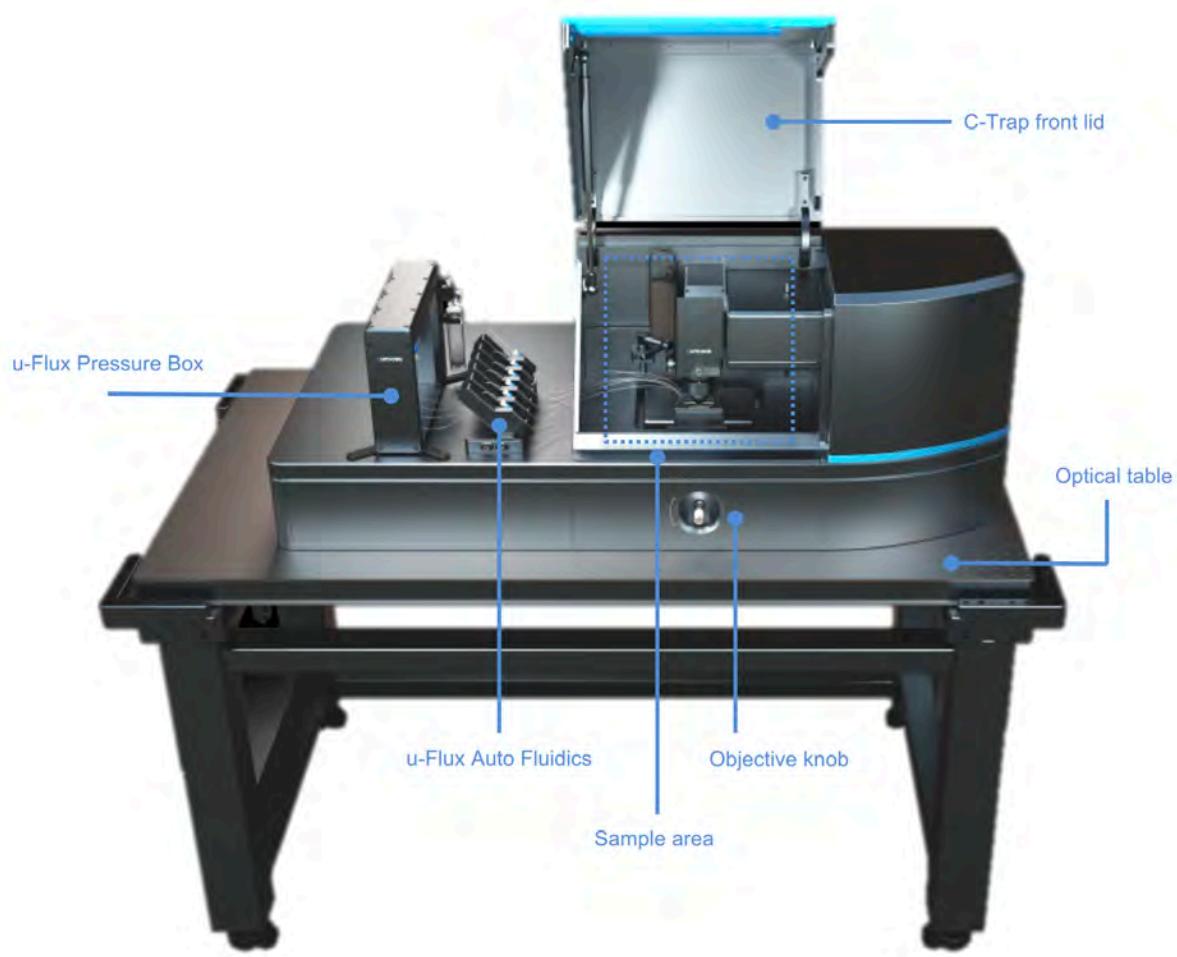


Figure 10.1.1: Overview of the C-Trap instrument.

10.2 Electronics rack

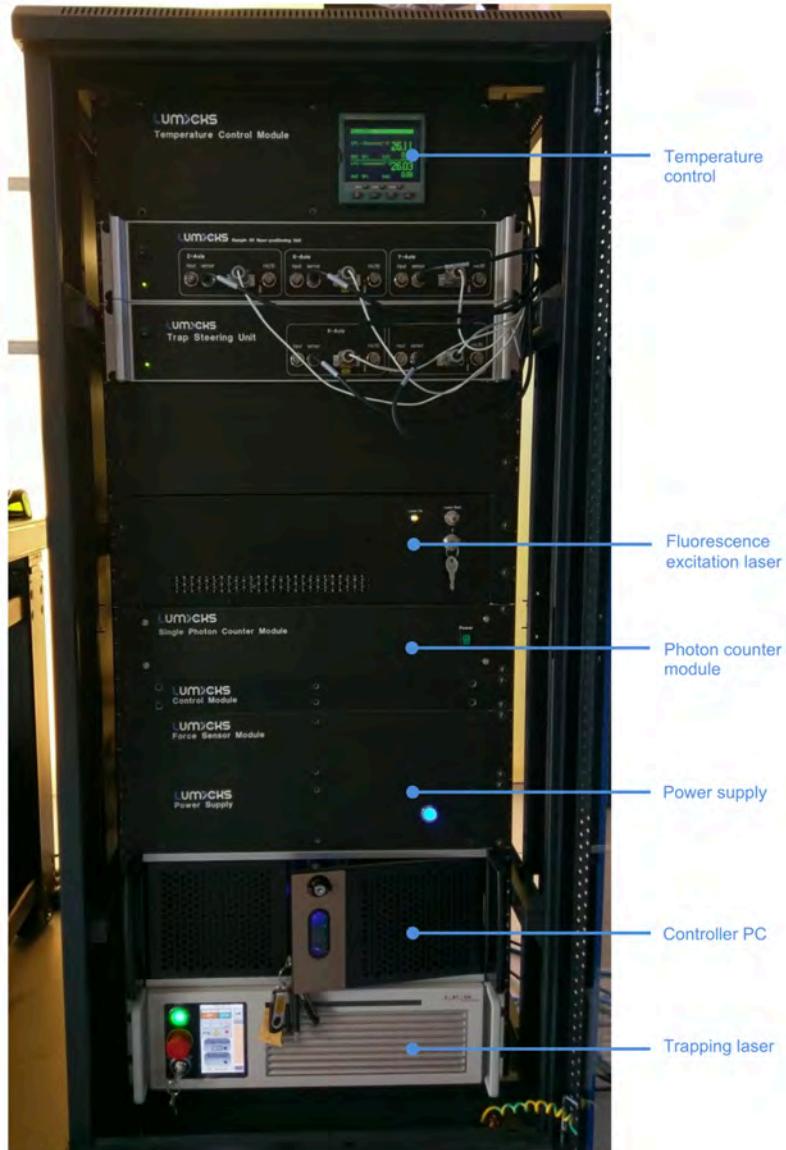


Figure 10.2.1: Overview of the C-Trap electronics rack.

10.3 Workstation

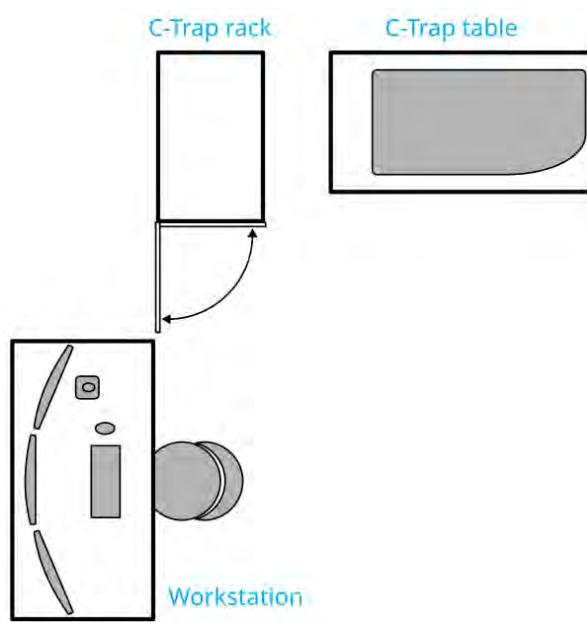


Figure 10.3.1: Recommended room layout for the C-Trap and workstation.

11 Basic operations

This guide provides a step-by-step overview of the basic operations including the startup process to prepare the system for conducting experiments.

Before proceeding with the operational steps, it is crucial to thoroughly review the safety instructions outlined in sections *General safety, maintenance and service, Laser safety, Cautions and notes*.

11.1 Starting up of the system and software

1. Switch on the electronics rack via the main power button located on the front panel.
2. Turn the key switch on the Trapping laser.
3. Power on the Controller PC and log in using your user account.
4. Locate the **Bluelake** [Figure 11.1.1](#) on the desktop and double-click to launch the software.

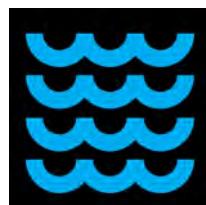


Figure 11.1.1: Bluelake software icon.

5. Select the desired user name, profile and configuration file.
6. Bluelake shows a splash screen and will then open on both screens (see [Figure 11.1.2](#) and [Figure 11.1.3](#))

Note: During the system starting up, you may hear loud, sharp noises. These noises are generated by motors and actuators inside the C-Trap instrument as they are homing; this is normal behavior.



Figure 11.1.2: Bluelake after starting showing the data visualization window (left screen).

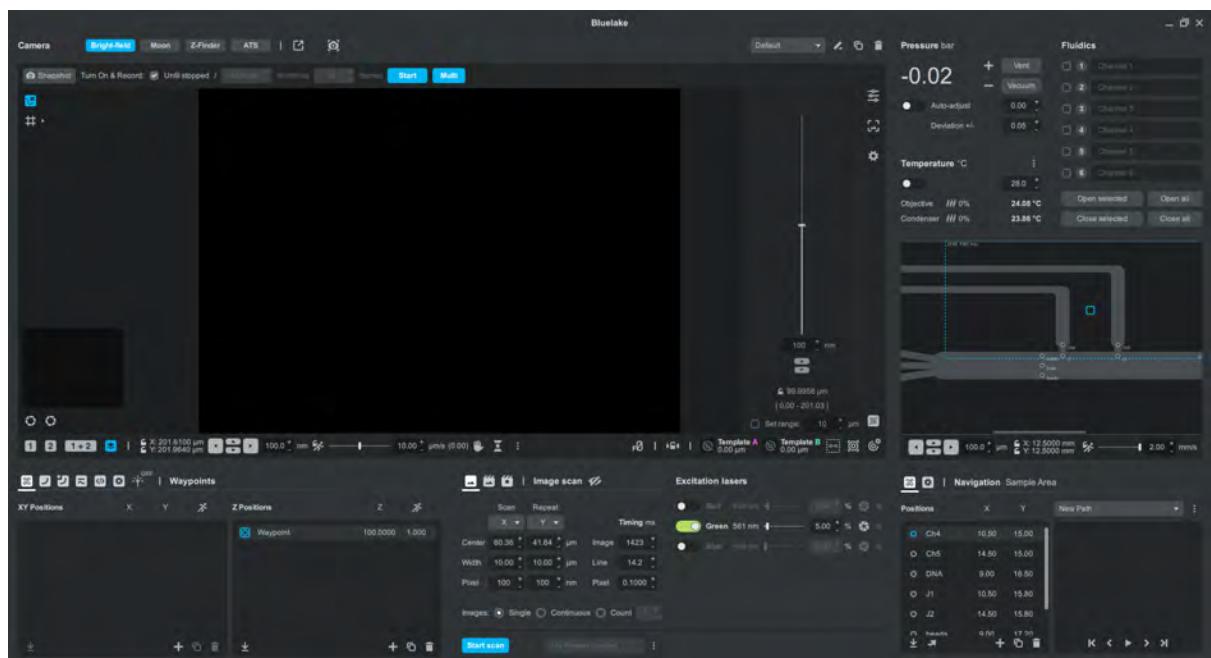


Figure 11.1.3: Bluelake after starting showing the control window (right screen).

11.2 Homing the sample stage

In certain instances, Bluelake may prompt you to home the sample stage. This can occur, for example, when power-cycling the C-Trap. Bluelake will automatically detect when the sample stage needs homing and will notify you accordingly during the startup process.

Note: It is important to home the sample stage when prompted. Failure to do so may pose a risk of damaging the microscope objective while moving the stage.

Upon selecting **Home Stage Now**, a dialog box will appear (see Figure 11.2.1), providing step-by-step instructions to guide you through the homing procedure.

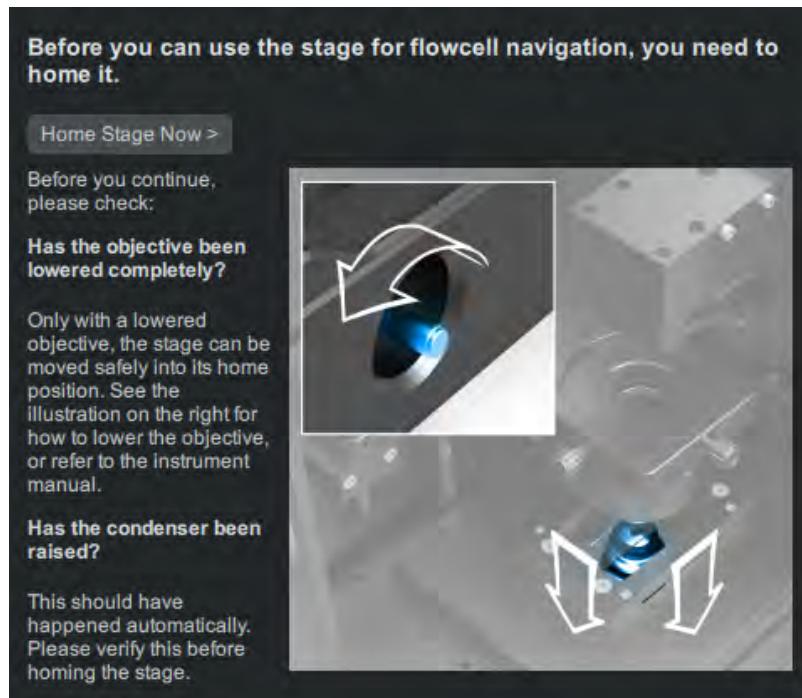


Figure 11.2.1: Sample stage homing dialog.

It is crucial to carefully follow these instructions. In particular, before initiating the homing procedure, ensure that the microscope objective is lowered, and the condenser is raised (see Figure 11.2.2). Neglecting to perform these steps may result in severe damage to these optical components.

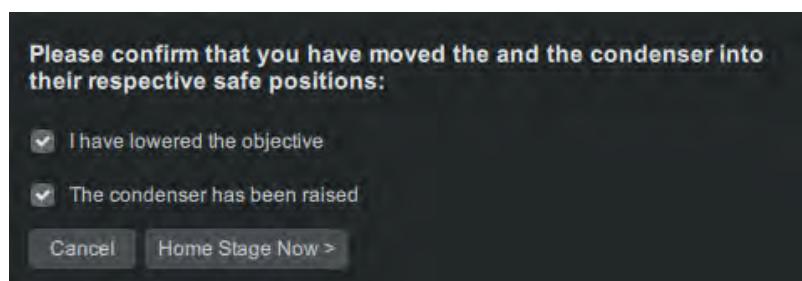


Figure 11.2.2: Confirmation dialog before stage homing.

You can repeat the microscope stage homing procedure at any time by accessing the **Calibration** menu in the main window of Bluelake and selecting **Home Stage**.

11.3 Adjusting the microscope objective correction collar

When conducting experiments using large beads ($>2.5\text{ }\mu\text{m}$ in diameter), it is recommended to set the correction collar of the microscope objective to the thickness of the coverslip or the bottom glass layer of the flow-cell (175 μm). This adjustment optimizes the confocal performance for such experiments.

However, trapping smaller beads ($<<2.5\text{ }\mu\text{m}$ in diameter) can be difficult with these collar settings. To improve the trapping stability, the correction collar can be adjusted to a value approximately 15% to 20% below the actual coverslip thickness. It is important to note that while this adjustment can improve trapping performance, the confocal performance may not be optimal, resulting in a decrease in resolution of up to 20%.

Note: Care should be taken when adjusting the correction collar to avoid excessive force or misalignment.

Note: For experiments conducted at an elevated temperature of 37°C using the oil immersion objective (TIRF), there is a dedicated correction collar scale specifically for this purpose. LUMICKS recommends referring to this scale when performing experiments under elevated temperature conditions.

To adjust the objective correction collar, follow these steps:

1. Remove the flowcell holder to gain access to the microscope objective.
2. Locate the scale setting of the correction collar (see [Figure 11.3.1](#)).
3. Adjust the correction collar to the preferred position; refer to the [Table 11.3.1](#) below for guidance on the correction collar setting.

Table 11.3.1: Overview of recommended correction collar settings.

Sample (thickness)	Bead diameter	Recommended setting
LUMICKS flow cell (0.175 mm)	Large ($>2.5\text{ }\mu\text{m}$)	0.175
	Small ($<<2.5\text{ }\mu\text{m}$)	0.15
Custom coverslip (#1.5; 0.17 mm)	Large ($>2.5\text{ }\mu\text{m}$)	0.17
	Small ($<<2.5\text{ }\mu\text{m}$)	0.15
Custom coverslip imaged at surface	Small ($<<2.5\text{ }\mu\text{m}$)	0.15



Figure 11.3.1: Water immersion objective used in the C-Trap (left) and TIRF oil immersion objective used in the C-Trap (right).

11.4 Adding immersion liquids for the microscope objective and condenser

Before starting an experiment, it is essential to apply immersion liquid onto the microscope objective and immersion oil for the condenser (see [Figure 11.4.1](#)).

The **objective knob** allows adjustment of the position of the objective lens relative to the sample. By rotating the knob, you can move the objective up or down ([Figure 11.4.2](#)).

Follow the instructions below for adjusting the objective position:

1. To move the objective upwards, rotate the objective knob in a clockwise direction. This will result in the objective approaching the sample.
2. To move the objective downwards, rotate the objective knob in a counter-clockwise direction. This will cause the objective to move away from the sample.

To properly add immersion liquids on the objective and immersion oil for the condenser, follow these steps:

1. Ensure that the trapping laser is turned off, the microscope objective is retracted, and the condenser is raised.
2. Slide the flowcell holder out to make the microscope objective tip easily accessible.
3. Place a drop of ultrapure water (max. 70 µl) on top of the microscope objective front lens.

Note: Depending on factors such as humidity, temperature, and laser power conditions, the immersion water may evaporate within 1 to 3 hours. It is important to prevent the immersion water from completely drying up as it may leave stains on the bottom of the flowcell. As a guideline, it is recommended to regularly refill the immersion water throughout the day. We

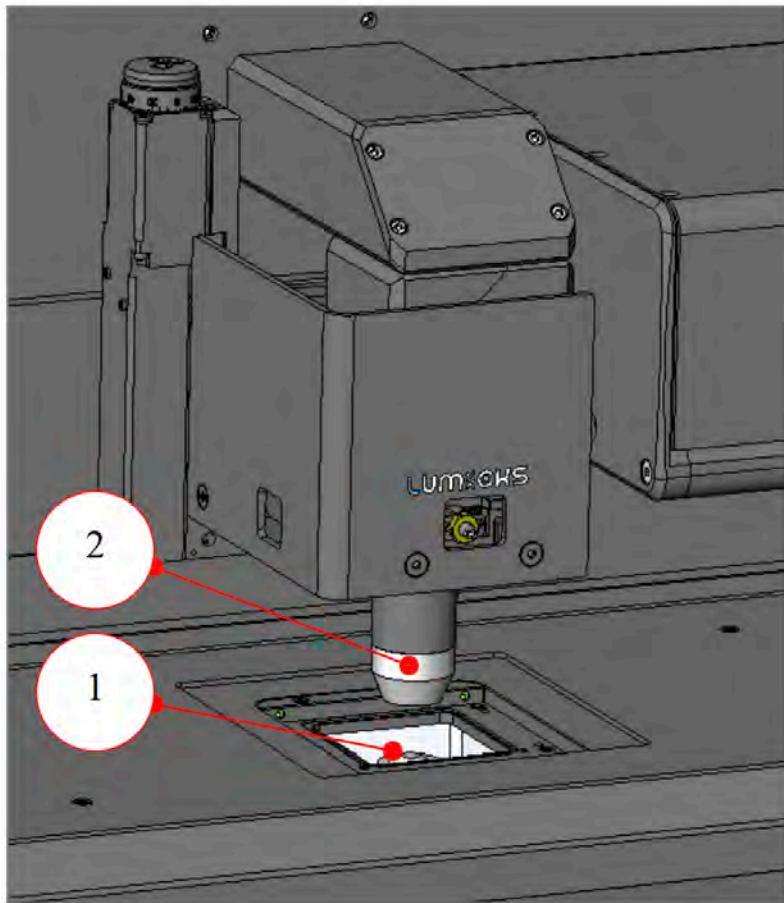


Figure 11.4.1: Sample area. 1: Objective. 2: Condenser.

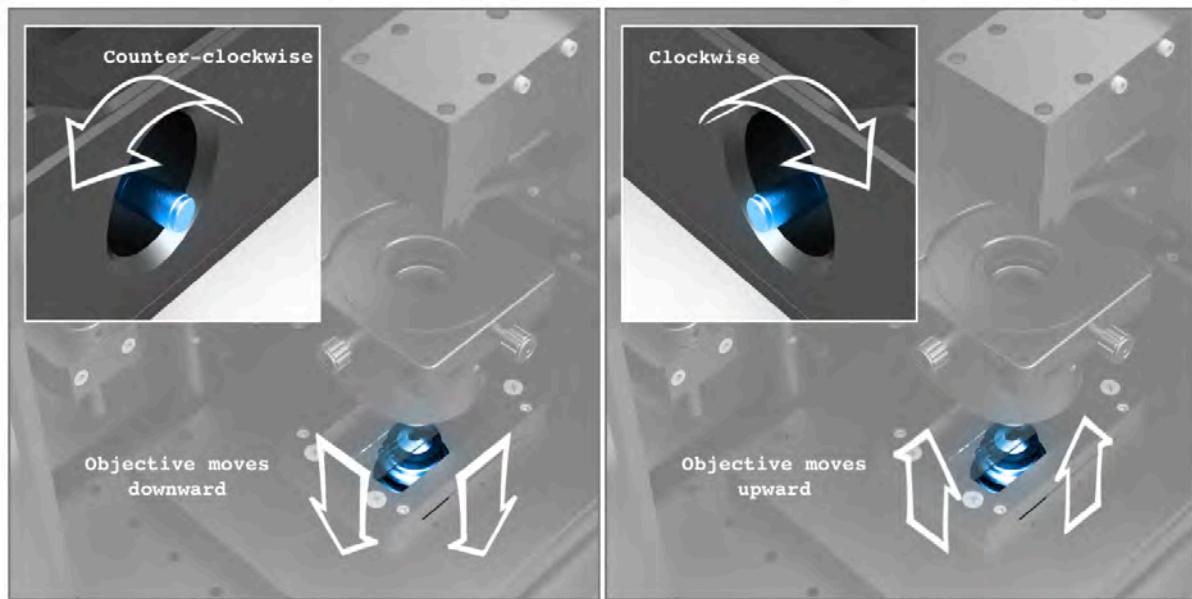


Figure 11.4.2: Moving the microscope objective downwards (left) and upwards (right) using the objective knob.

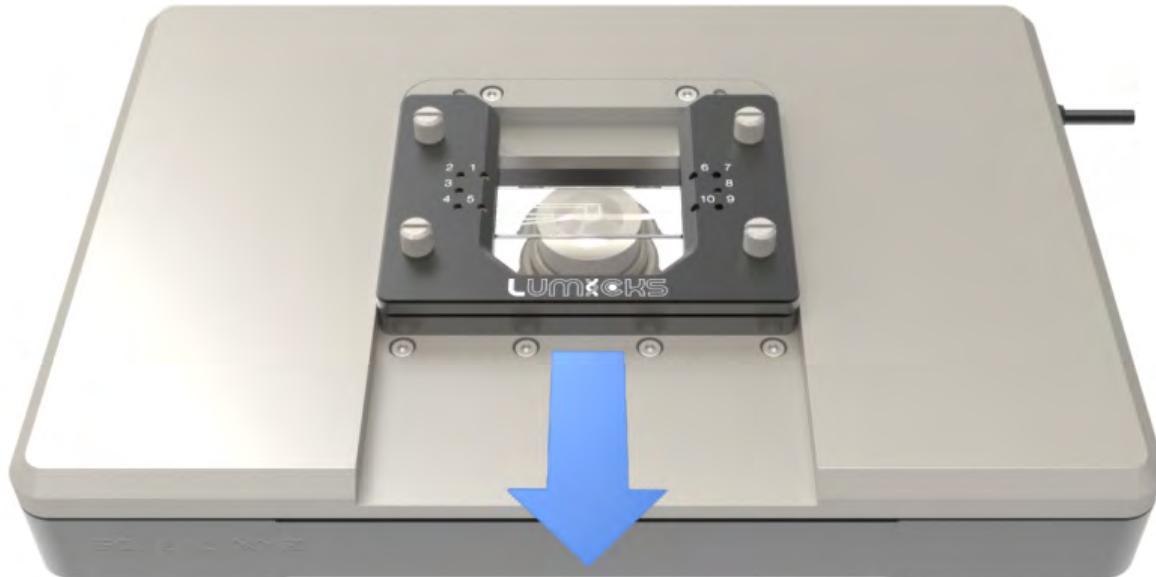


Figure 11.4.3: Removing and placing the flowcell holder.

recommend refilling the immersion water every 2 hours during experiments.

4. Slide the holder back into its snapping position, securing the flowcell in place.
5. Move the objective up as detailed in section 11.4 above, until the water droplet makes contact with the bottom of the flowcell. See figure 10.
6. Perform a visual inspection to confirm that the microscope objective is positioned below one of the microfluidic channels. It is important to avoid focusing the laser on an edge or a non-channel area of the flowcell when the laser is ON, as this can potentially damage the optics and the flowcell.
7. Add a drop of immersion oil for condenser on top of the flowcell.
8. Close the lid of the C-Trap.

11.5 Turning on the trapping laser

To activate the 1,064-nm trapping laser emission, follow these steps:

1. Reset the laser by pressing the red **RESET** button and turning it clockwise until the knob releases towards you (see [Figure 11.5.1](#)). Then press the green **START** button, which should illuminate to indicate that the power supply is running. This step is necessary after opening the lid, as the safety interlock turns off the laser.

Note: Whenever the lid is opened, the safety interlock will deactivate the laser. Repeat this procedure to reset the laser.

2. In the bottom left corner of the Bluelake control window (right screen), locate the **Power Control** center (see [Figure 11.5.2](#)). Click on the gear button to access the settings. Set

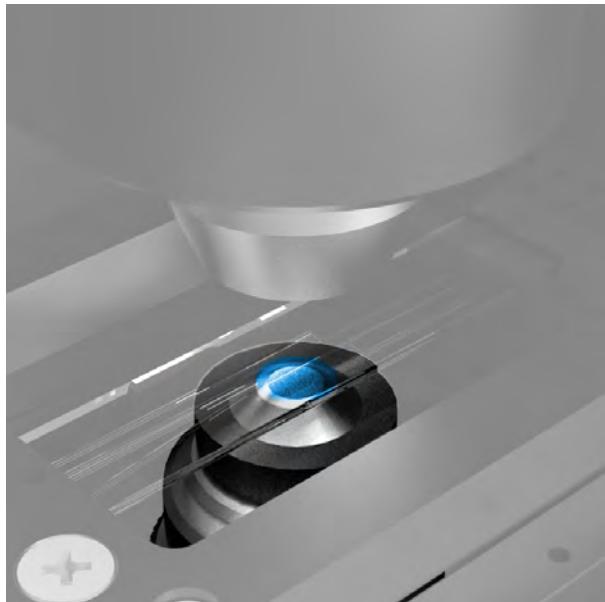


Figure 11.4.4: Carefully bring the microscope objective into contact with the sample, ensuring that the immersion liquid drop makes contact with the glass flowcell.



Figure 11.5.1: Trapping laser rest and start buttons.

the **Trapping Laser** power to 100% to enable laser emission (confirmation will be required before activation). Ensure that the **Overall Trapping Power** is also set to 100%. The power for the Bright-field LED should be turned off when adjusting the objective position.

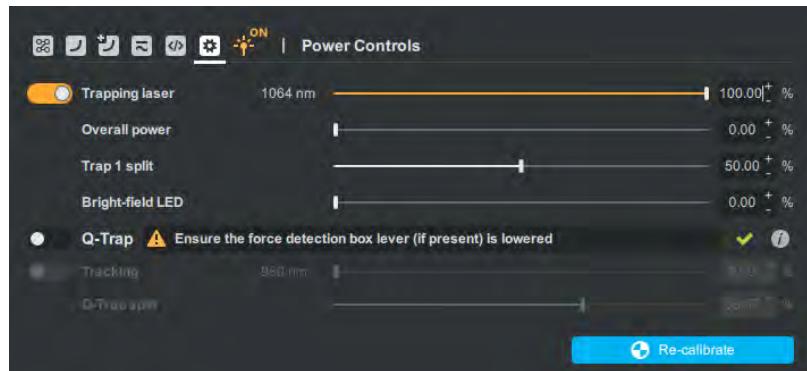


Figure 11.5.2: Power Controls panel in Bluelake.

11.6 Finding the correct z-position of the microscope objective

To determine the correct Z-position of the microscope objective, follow these steps using the reflection of laser light from the glass flowcell. Typically, you want to position the focus of the objective in the middle of the channel of the flow cell, approximately 50 µm above the lower surface.

Note: If your C-Trap is equipped with a nanostage, verify that it is at a z-position that will allow you sufficient range for your experiment. Larger changes in the nanostage z-position might require repeating the microscope objective and condenser setup.

1. Ensure that the flow cell channel is filled with water or aqueous buffer solution.
2. Select the Z-Finder camera tab in Bluelake. Set the camera exposure to the maximum value (99.9 ms). This camera displays the light reflected by the sample, and at this step, you should be able to observe some stray light in the Z-Finder camera.
3. If your system is equipped with a nanostage: In the **Trap steering** section of the software, select **Nanostage** and move the nanostage to the 100 µm position (middle of the range) in z-direction.



Figure 11.6.1: Trap steering and nanostage controls.

4. While observing the camera image, carefully and slowly rotate the **objective knob** in a clockwise direction to move the objective upward. Be extremely cautious to prevent the objective front from making contact with the flow cell, as it can cause damage.

As the trapping laser beam focuses on the interface between the glass and water (or immersion liquid), it will be reflected, resulting in the appearance of concentric interference rings. These



Figure 11.6.2: Z-direction controls for the nanostage.

interference rings will expand and contract symmetrically as the objective gets closer to the flowcell.

Note: There may be a constant interference pattern present that does not change with the objective position. This pattern originates from the reflection of the back aperture of the microscope objective and should be disregarded.

The variable interference rings will come into view and disappear as the objective is brought closer to the flowcell. The first pattern corresponds to the interface between the immersion liquid and the external surface of the flowcell. The second pattern corresponds to the interface between glass and water, which stems from the bottom of a water-filled channel in the flowcell. The third pattern corresponds to the interface between water and the top of the flowcell.

Note: When using the TIRF objective with oil immersion, the reflection from the glass-oil interface (external surface of the flowcell) will be significantly weaker than the reflection from the glass-water interfaces of the water-filled channel in the flowcell, making it easier to detect and distinguish between the two.

5. Adjust the microscope objective to focus on the third reflection position.
6. Now, move the objective back to a position that is halfway between the second and third reflection positions. This is where the reflection rings transition from expanding to contracting. Alternatively, if your system is equipped with a nanostage, you can use the software to adjust the Z-position. In the **Trap steering** section of Bluelake, select **Traps**



Figure 11.6.3: Cross-section of the LUMICKS flowcell. The correct Z-position of the objective is located between the 2nd and the 3rd reflection.

Stage and move the nanostage to 50 μm .

7. It is crucial not to bring the microscope objective closer to the flowcell than the position where the third interference pattern is observed. Moving beyond this point can result in damage or breakage of both the microscope objective and the flowcell.
8. Once you have determined the appropriate microscope objective position, you can set the laser power to 30% if you need to determine the condenser position, or 100% if you have already configured the condenser height for your specific experiments.

11.7 Finding the correct z-position of the condenser

Ensure that your **Trapping laser power** is set to 30% and the **Overall power** to 20% (or lower, in case of a more sensitive sample)

If you are using a Q-Trap, switch to dual-trap mode by setting the **Q-Trap split** to 0% and the **Tracking laser power** to 0%. Also, make sure that the force detection is manually switched to dual trap detection (refer to the Appendix for more details).

Note: If your C-Trap is equipped with a nanostage, verify that it is at a z-position that will allow you sufficient range for your experiment. Larger changes in the nanostage z-position might require repeating the microscope objective and condenser setup.

Most C-Traps are equipped with a motorized remote-controlled condenser which can be operated following the below procedure:

1. Open the condenser control (drop down from **Tools** menu, or available after toggling the “Moon” camera).
2. Click the **Approach** button to lower the condenser to a safe position (see [Figure 11.7.1](#)).
3. Toggle the auto-adjust box under the **Image Adjustment** settings on the top right of the “Moon” camera image window.

4. Set the step size to 100 μm , then click the **Step down** button several times to gradually lower the condenser while watching the image. You should see an expanding moon-like circle in the “Moon” camera view with each step down.
5. Continue lowering the condenser until it touches the oil (visible event on the camera) or until the diameter of the lit area approaches the one from the “good moon” image on the desktop.
6. Fine-tune the position by setting the step size to 10 μm and continuing to step down until the number of horizontal stripes and their overall patterns (curvature and thickness) match the “good moon” reference image on the Windows desktop.
7. Stepping down should decrease the number of bands while stepping up should increase them. If the reverse occurs, this likely indicates that you are too close to the flowcell and you should immediately raise the condenser.

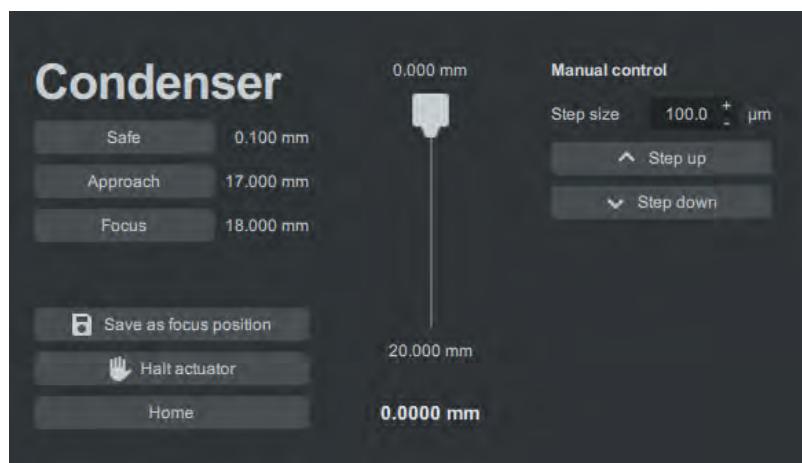


Figure 11.7.1: Condenser control window.

8. Restore the **Trapping laser power** to 100% and the **Overall power** to the desired power.
9. The stored condenser focus position will become invalid whenever the flowcell is remounted, the microscope objective is remounted, or the (optional) nanostage is set to a different height. In these cases, the above procedure needs to be repeated to find the correct Z-position of the condenser. However, if none of the previously mentioned components in step 9 are changed, you may store this correct condenser position by clicking the **Save as focus position** button. This allows you to quickly move to that position again by using the **Focus** button.

Figure 11.7.2 shows examples of a properly aligned condenser position and incorrect positions.

If your C-Trap is equipped with a manual condenser actuator, follow these instructions below to bring the condenser in contact with the sample.

1. Turn off the 1,064 nm trapping laser and open the C-Trap lid.
2. Put your right hand under the condenser holder while avoiding to touch the condenser lens. Gently and steadily lift the holder off its stopper to feel its weight.
3. While your right hand firmly holds the weight of the condenser, use your left hand to rotate the condenser locking arm to the left to release it from its resting position (see Figure 11.7.3).

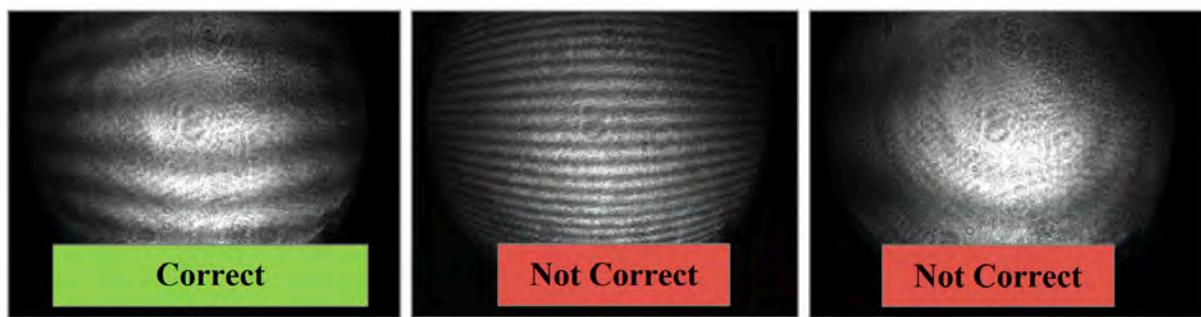


Figure 11.7.2: Example “Moon” images resulting from correct and incorrect condenser positions.

4. Slowly and gently lower the condenser with your right hand until it reaches the stopper. Releasing the condenser abruptly may cause damage to the optics.
5. Close the lid, turn on the 1,064 nm laser, and look at the “Moon” camera image. Verify that the number of horizontal stripes and their overall patterns (curvature and thickness) match the “good moon” image on the desktop
6. In case the images do not match, follow one of the two workflows below:

Manual condenser workflow 1:

1. To increase or decrease the number of stripes visible in the moon image, adjust the height of the **objective** very slightly while looking at the “Moon” camera. If more than 1/8 turn of the objective is needed, follow the instructions of workflow 2. Rotating the **objective** micrometer clockwise should reduce the number of stripes.

Manual condenser workflow 2:

1. Turn off the 1,064 nm trapping laser, and open the lid. Rotate the condenser micrometer slightly clockwise to raise the condenser (increase the number of stripes). To orient the clockwise direction, use your right hand thumb pointing up vertically. Your fingers then naturally point towards the “clockwise” direction as viewed from the below. The other direction will lower the condenser (and decrease the number of stripes).
2. Close the lid and turn the 1,064 nm trapping laser back on.
3. Compare the “Moon” camera image with the reference “good moon” reference image. If they still do not match, repeat steps 1-3, but adjust the condenser up or down accordingly, until the “Moon” camera image matches the “good moon”.

Once the condenser position is set (and the “moon” image matches the reference well), restore the **Trapping laser power** to 100% and the **Overall power** to the desired power.

Note: Avoid rapidly lowering the condenser and making sudden contact with the immersion oil to prevent the formation of air bubbles. Air bubbles in the immersion oil can disrupt the experiment, leading to drift and additional noise in the force signal.

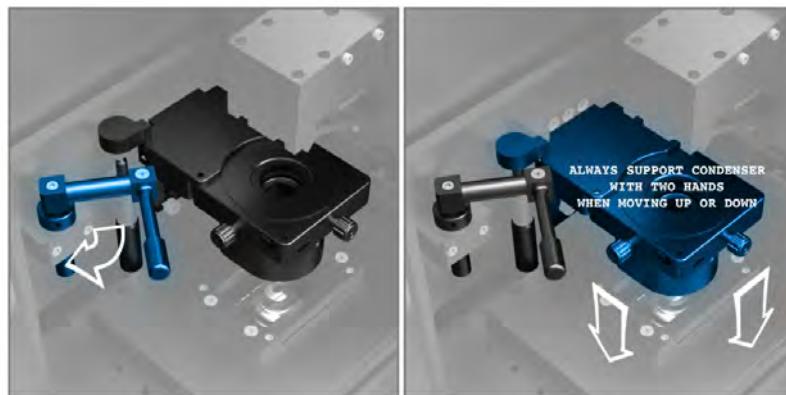


Figure 11.7.3: Releasing and lowering the manual condenser.

11.8 Flowcell selection & visualization

In Bluelake, you can easily select and visualize different flowcell designs without restarting the software (see [Figure 11.8.1](#)). The detailed flowcell geometry is displayed, allowing you to match the flowcell map with the actual position of the flowcell captured by the camera.

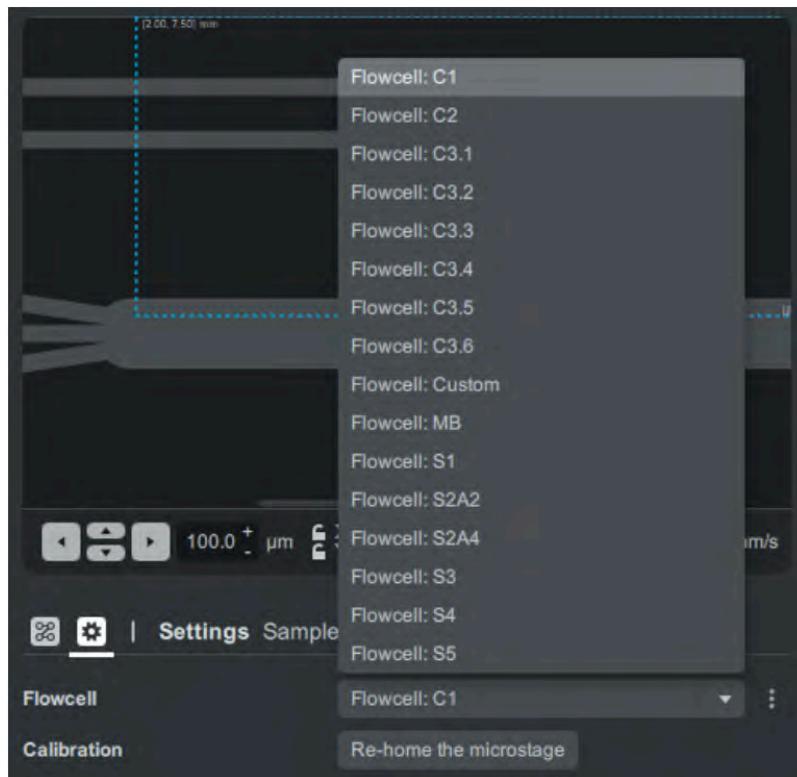


Figure 11.8.1: Dialog to select the flowcell type in Bluelake.

To align the flowcell map with the physical flowcell position, follow these steps:

1. Right click on the corresponding position of the physical flowcell shown in the Bluelake flowcell map (see [Figure 11.8.2](#)).
2. A message will appear, reading **Set as current microstage position**.
3. Right click on the message to confirm and set the selected position as the current mi-

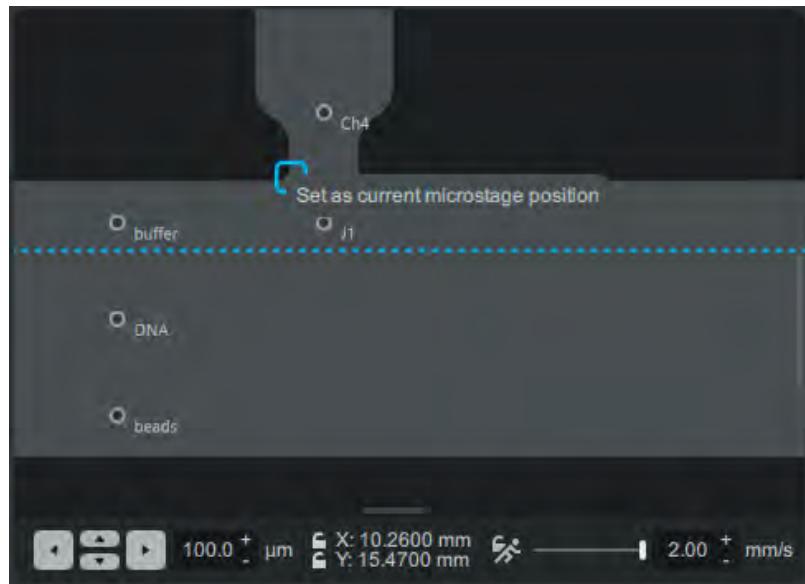


Figure 11.8.2: Set current microstage position dialog.

crostage position.

This allows you to accurately match the flowcell map with the physical flowcell.

11.9 Force calibration

The following instructions describe how to perform a force calibration.

1. Trap beads.
2. Open the **Force calibration** window by pressing the F9 key or the **Re-calibrate** button in tab **Power Controls** (see [Figure 11.9.1](#)).
3. Enter values for bead sizes, temperature, fluid viscosity and density, points per block, measurement time and fit range.
 - a. If working far away from the flowcell surface (ratio of height to bead diameter > 5), leave **Close to surface** unchecked. Choose **Hydrodynamic correction** and, if available, choose **Active calibration** and press **Measure** to start the calibration.
 - b. If working close to the flowcell surface (ratio of height to bead diameter < 5), choose **Close to surface** to get to height determination (see [Figure 11.9.2](#)). To determine the height, choose **Auto**, check the correct microscope objective type and press **Touchdown** to start height determination. If the ratio of the determined height and the bead diameter is smaller than 0.75, calibrating with checked **Hydrodynamic correction** is not possible, so uncheck it. If available, choose **Active calibration** and press **Measure** to calibrate. Note that height determination requires a bead in Trap #1.
4. Press **Apply** to apply calibration on selected force channels.

Note: For more guidance on when to use which calibration type, refer to the [Force calibration technical note](#).

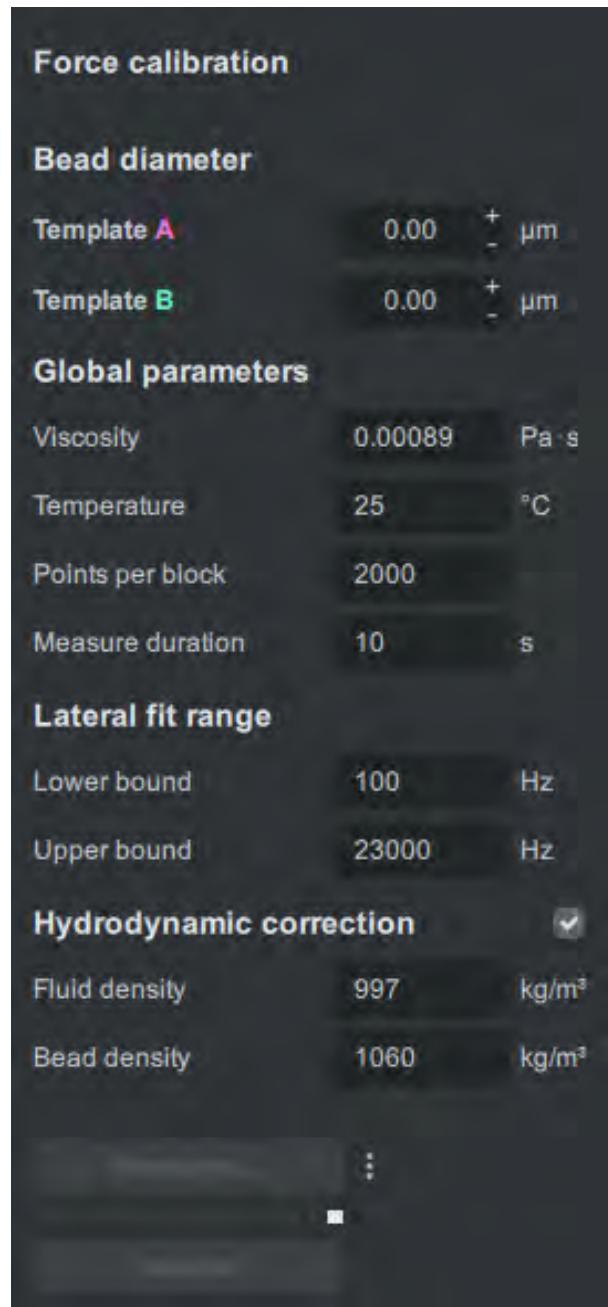


Figure 11.9.1: Dialog to for the force calibration in Bluelake.

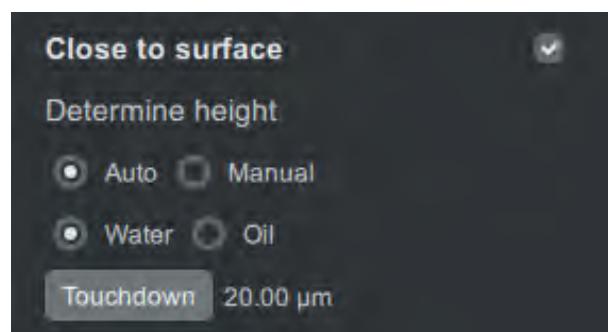


Figure 11.9.2: Feature to take surface effects into account in the force calibration in Bluelake.

11.10 Fluorescence excitation lasers and confocal scan settings (C-Trap Dymo)

To set up the fluorescence excitation lasers and configure the confocal scan settings in a C-Trap Dymo, follow these steps:

1. Switch ON the single photon counting module located in the electronics rack adjacent to the C-Trap. If it is already on, the button will be lit.

Note: Do not open the C-Trap lid while the single photon counting module is switched on. Switch off the module before opening the lid to avoid damaging the electronics.

2. Switching on the lasers:
 - a. Go to the **Excitation Laser Control** panel in Bluelake and select the checkboxes next to the appropriate color (wavelength) of the excitation laser required to excite your sample. You can select multiple checkboxes to image multiple fluorophores simultaneously.
 - b. Set the desired excitation power for each laser by adjusting the slide-bar or typing the desired value in the percentage box.
 - c. Click and select the **Sync** box to ensure that the shutter opens only when you start the imaging. Alternatively, you can click and select the **Shutter** box to keep the shutter always open.
3. Select the region of interest (ROI): Use the ROI icon in the image screen to select and draw a box around the area you want to scan for fluorescence imaging.
4. Choose the appropriate scan settings:
 - a. Navigate to the **Scan Settings** panel, which includes three tabs: **Image**, **Kymograph**, and **Point** (see [Figure 11.10.1](#)).
 - b. The **Image** tab displays the scanning dimensions on the X and Y axes. Select the appropriate settings for the scan, including the fast axis direction.
 - c. The **Kymograph** tab allows you to perform a series of 1D scans to create a kymograph. Choose the fast axis and adjust the scanning settings to optimize imaging efficiency and prevent sample bleaching while obtaining a high signal-to-noise ratio. The **Point** tab enables you to perform a point scan, which can be useful for positional bleaching experiments or techniques like fluorescence correlation spectroscopy (FCS) or fluorescence resonance energy transfer (FRET).

11.11 Super-resolved fluorescence imaging (C-Trap Dymo 700)

To activate super-resolved fluorescence imaging, please follow these steps:

1. Turn on the depletion laser in the electronics rack by turning the key to **1** position on the front panel.
2. The **Excitation Lasers** panel in Bluelake for the C-Trap Dymo 700 allows control over the depletion laser (see [Figure 11.11.1](#)). Check the box corresponding to this laser and set its power to the desired level using the slider.

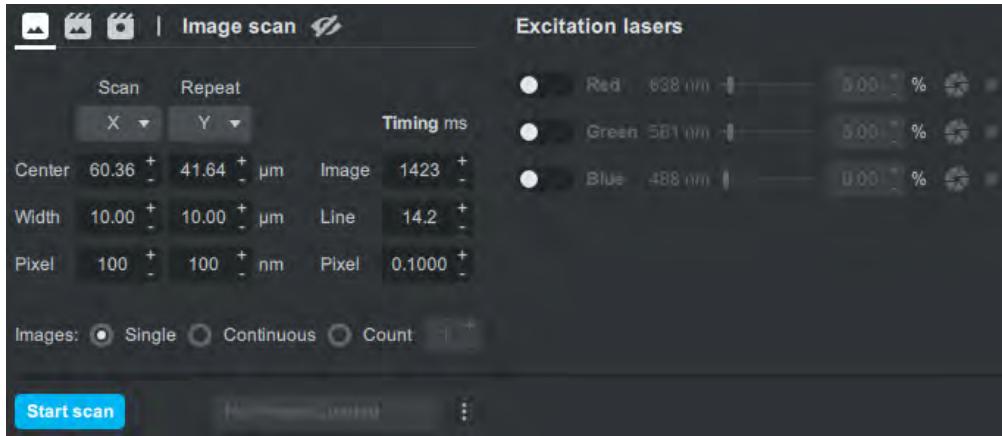


Figure 11.10.1: Figure: Image scan and fluorescence excitation laser settings panel.

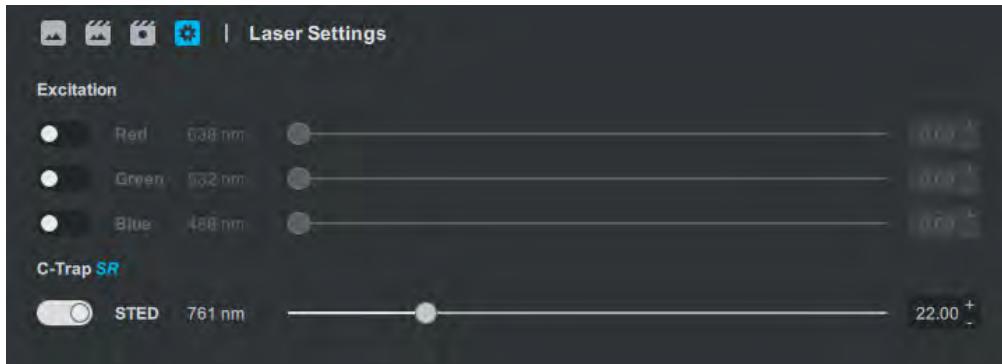


Figure 11.11.1: Figure: Turning on STED super-resolved fluorescence imaging.

3. Perform scans in a similar manner as the confocal mode.

Note: The super-resolved fluorescence imaging is compatible only with the 638-nm (red) excitation laser. Furthermore, the red excitation laser of the C-Trap Dymo 700 operates in a pulsed mode at 1 MHz repetition rate. In the confocal module, the red excitation laser operates in continuous wave mode.

11.12 Fluorescence excitation lasers and Widefield/TIRF camera settings (C-Trap Edge)

1. Make sure the lid of the C-Trap is closed, the fluorescence lasers do not switch on when the lid is open.
2. Identify the excitation laser module in the electronics rack (see [Figure 11.12.1](#)).



Figure 11.12.1: Figure: The two types of laser modules in the C-Trap without (top) and with (bottom) key switch.

If the module has a key and a button (bottom picture): Switch on the fluorescence excitation laser module located in the rack adjacent to the C-Trap. Turn the key clockwise from 0 to 1 and press the **Laser Start** button.

Note: The indicator light should be lit if the fluorescence excitation laser module is on. It may take some time for all lasers to be recognized. Please wait for approximately 30 seconds for the laser module to warm up.

3. Switch on the lasers in Bluelake:
 - Go to the **Excitation** window in Bluelake (see [Figure 11.12.2](#)). You will find the fluorescence excitation laser controls with toggles on the left that allow you to enable the lasers.

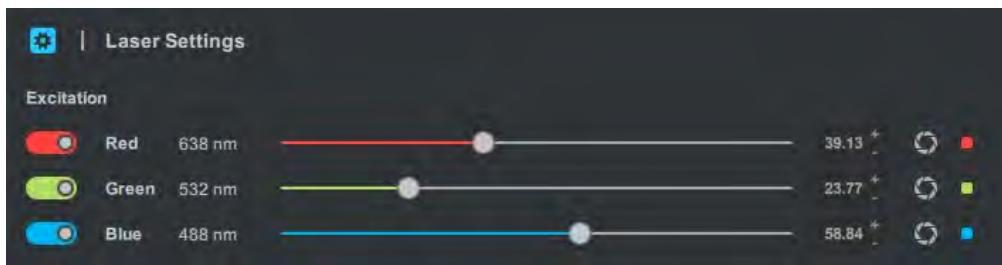


Figure 11.12.2: Figure: Fluorescence excitation laser panel and shutter control.

Note: There is a warm-up time required for the lasers to be enabled. Additionally, when the lasers are enabled (left toggles ON), a very small residual light will reach the sample. This value is measured and available in the SAT document.

- Set the power of each enabled laser to the desired percentage for your experiments. You can adjust the power by sliding the slide bar to the desired value or by typing in the numerical input field and pressing enter.

Note: The power shown represents the direct output power (%) of the laser, not the actual power in the sample (mW). Refer to the SAT document for the actual power (mW) at the microscope objective.

- On the right side, you will find a manual control that emits the selected percentage output (see [Figure 11.12.2](#)). These right toggles are used to turn the fluorescence excitation laser on and off while operating, ensuring continuous stability while the lasers are still enabled using the left toggles.

3. Configuring the Widefield/TIRF camera settings:

- Go to the camera window to access the Widefield/TIRF camera image labeled **WT** and configure the acquisition settings (see [Figure 11.12.3](#)). The main window contains several toolbars with different settings.

Note: If the camera image appears to only partially display an image, you can adjust this by using the camera region of interest (ROI) button located at the top.

- Drag the blue bounds that appear in the image to adjust the camera ROI. A smaller horizontal width of the camera ROI allows for faster camera rates.
- You can disable different color channels for display in the main camera window by clicking the colored **R**, **G**, or **B** boxes in the top right corner. On the left side, you can enable/disable the Mini Map of the camera view, enable/disable the grid, and specify the grid size and color using the small arrow.
- The toolbar on the right side contains several settings:
- The first setting is **Image Adjustment** (see [Figure 11.12.4](#)). This panel allows you to adjust the intensity of colors shown on the camera based on their pixel values. You can manually adjust the color detection channels' histograms by selecting the **R**, **G**, or **B** boxes and using the colored range slider. Alternatively, you can enable the **Auto-adjust** box for

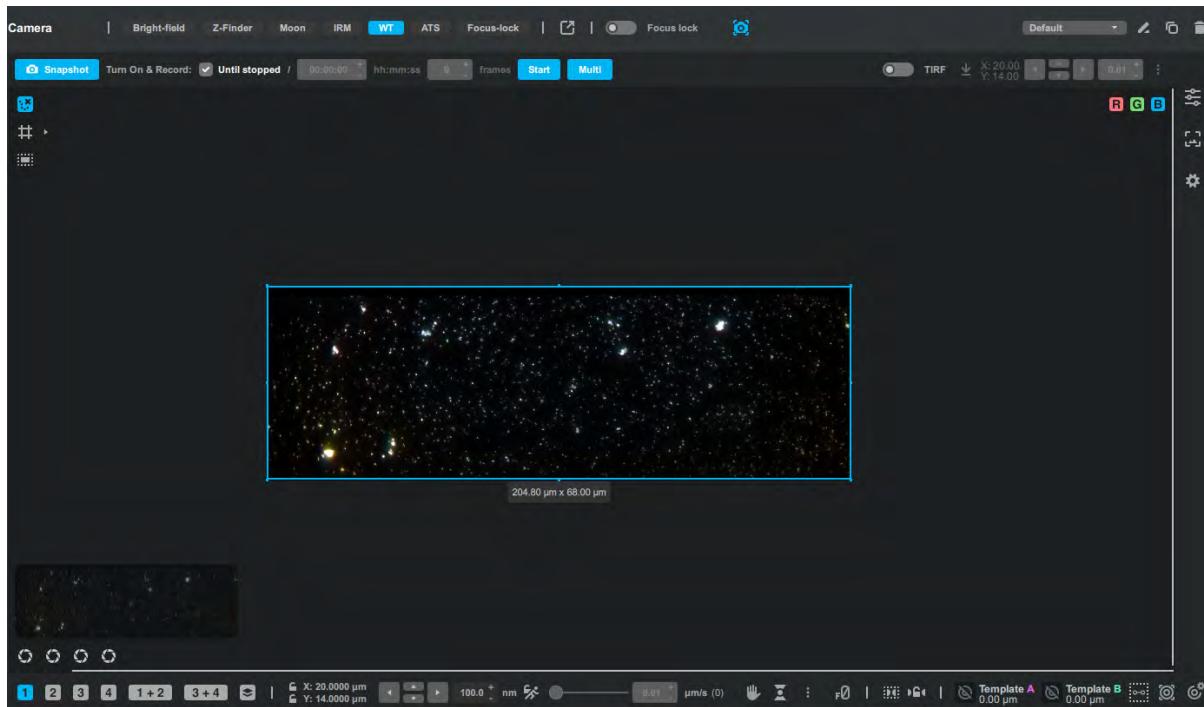


Figure 11.12.3: Figure: Widefield/TIRF camera image panel.

automatic adjustment. Once ticked, the user can set the percentage outliers that should be rejected in defining the left and right bound for the color display.

- Each color can also be displayed using a different color lookup table. This is done by enabling the **LUT** toggle and selecting the desired color lookup table. The camera window normally displays the default color channels overlapped in the main window. These color channels can be split to be displayed vertically in the main window by enabling the ***Split colors** toggle.

Note: Please note that the .tiff files exported by Bluelake are the unaligned, raw images. The correction factors are exported in the metadata, allowing easy post-correction using the LUMICKS Pylake package or your own data analysis scripts.

- If alignment is unsatisfactory, a calibration slide can be mounted, and a new alignment can be stored by pressing the cogwheel next to the toggle. (Please consult LUMICKS personnel on the steps if you are unsure on how to mount a calibration slide) From there, there is the option to **Snap & align** an image from the current camera image in focus on the calibration slide. Once done, a user can decide to press **Overwrite stored alignment** to overwrite with an updated alignment. The user can also **Restore factory alignment** to the setting that was set previously by LUMICKS personnel.
- The second setting in the right-hand side toolbar is **Camera Settings** (see Figure 11.12.5). This panel allows the user to adjust the exposure time and framerate of the camera. These values are intrinsically linked and will limit each other's values when set.
- The window also contains an **Exposure sync** setting. This setting automatically and continuously triggers the emission of the excitation synchronized to the exposure time of the camera, turning the lasers off when the camera is not exposing. For example, if the exposure is set at 500 ms and the framerate at 1 Hz, the lasers will be emitting for 500



Figure 11.12.4: Figure: Widefield/TIRF image adjustments panel in Bluelake.

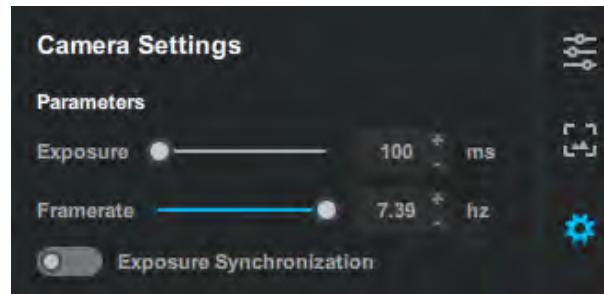
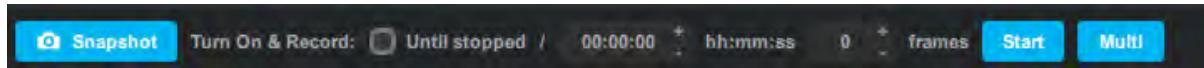


Figure 11.12.5: Figure: Widefield/TIRF camera settings panel in Bluelake.

ms only and will not be emitting during the rest of the time until a new camera frame is captured.

- The top bar in the camera view offers options for camera acquisition (see [Figure 11.12.6](#)). Going from left to right there is the **Snapshot** button that allows a user to take a single snapshot with the current camera settings. This data is saved as a .tiff file to the current data output folder set in Bluelake.



[Figure 11.12.6: Figure: Widefield/TIRF camera acquisition settings panel in Bluelake.](#)

- There are multiple settings for recording camera movies to the right. For the recording length of the movie the user has 3 options: **Until stopped** for continuous recording until aborted by the user, **hh:mm:ss** for a specified time length of the movie or a number of **frames** that the user wants to record at the current camera framerate. To start the recording, press **Start**.
 - If a user wants to set recording of multiple cameras simultaneously, this can be configured using the **Multi** button.
- To switch between the Widefield and TIRF modality, toggle the **TIRF** switch (see [Figure 11.12.7](#)). This will physically move the diffuser (in for widefield, out for TIRF) and adjust the TIRF mirror.

The angle of the TIRF mirror can be further finetuned by the user using the arrow keys. These values are quite small, and it is recommended to move by steps of ~0.01 in the y-direction to finetune. It is generally not recommended to tune the x direction more than 0.01 to the left or right from the standard position. If a user wants to save a new optimal position for TIRF, this can be done by pressing the **Save angle** button.



[Figure 11.12.7: Figure: TIRF illumination angle settings panel in Bluelake.](#)

- Restoring the TIRF angle factory settings as set by LUMICKS personnel can be done by clicking the three dots button.

Note: The TIRF modality requires the TIRF oil immersion microscope objective to be used and a TIRF-compatible sample with fluorophores at the sample surface (within ~100-150 nm of the interface).

11.13 IRM LED and camera settings (C-Trap Edge)

1. Select the IRM camera by choosing **IRM** in the camera window.
2. Turn on the IRM illumination LED in the top toolbar on the camera view.
3. Configure the IRM camera using the settings in the right toolbar and adjust the recording settings in the top toolbar (see [Figure 11.13.1](#)).
4. Typical exposure values are about 2-4 ms to avoid saturation of the IRM camera. Keep the **Gain** setting at 0% to minimize noise in the signal. Set the **Framerate** to 200 Hz for optimal recording.

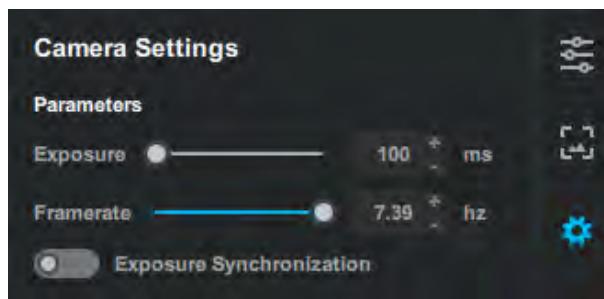


Figure 11.13.1: Figure: IRM camera settings panel in Bluelake.

5. To improve the IRM image quality, you can select the second option in the right toolbar and set the **Frames to average** to "10". This will result in a framerate of 20 Hz and improved signal-to-noise ratio (SNR) as each set of 10 frames is averaged on the camera.
6. For a cleaner IRM image and to remove surface or image irregularities, you can perform a background subtraction (see [Figure 11.13.2](#)) and specify a radius (10 µm or larger) over which the nanostage can move in a circular pattern. Multiple frames are recorded as the nanostage moves in this circle, and a background image is generated. This background image can then be subtracted from the normally acquired image to remove irregularities induced by the surface. Enable the **Background Subtract** toggle after acquiring an image to perform this background subtraction.

Note: Ensure that the nanostage has the range to perform a circular movement for background subtraction and is not close to the edge of its movement range (e.g., 0 or 200 µm in X, Y, Z).

11.14 Focus lock (C-Trap Edge)

1. Before turning on the focus lock, make sure to focus on the desired imaging surface. If you are using optical traps, allow the system to thermalize for at least 30 minutes.
2. To activate the focus lock, click on the **Focus lock** toggle in the camera window (see [Figure 11.14.1](#)).

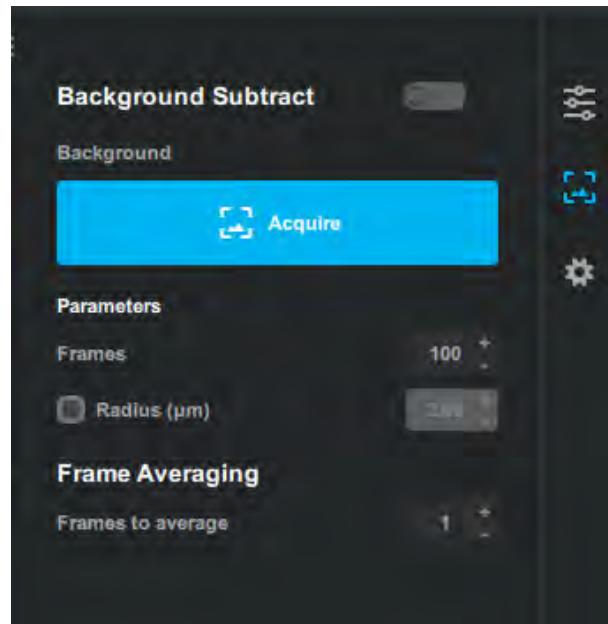


Figure 11.13.2: Figure: Background subtraction feature in Bluelake.

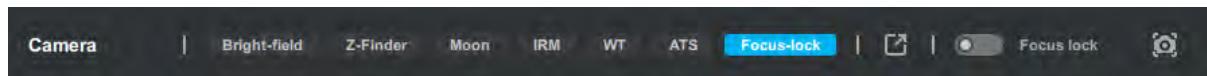


Figure 11.14.1: Figure: Focus lock toggle in Bluelake.

Note: The focus lock operates by tracking the reflection of a weak infrared laser spot that is reflected at the imaging surface. It is optimized for a glass-water interface, and it may be challenging to use with samples that have a weak reflection or strong light scattering. The focus lock primarily corrects for thermal drift in the system, which occurs over longer time scales (seconds to minutes).

3. The focus lock tracking signal and the applied corrections can be monitored in the timeline window.
4. To deactivate the focus lock, click again on the **Focus lock** toggle.

Note: If you intend to intentionally move the nanostage in the Z-direction while the focus lock is active, be aware that the system will continuously attempt to return to the previous focus position. If you need to update the locked Z-position, simply turn off the focus lock, move to the desired Z-position, and then reactivate the focus lock.

11.15 Q-trap settings (C-Trap Dymo 400, Dymo 700 and Edge 450)

When using the Q-trap mode, please follow these steps:

1. Switch on Q-Trap mode using the **Q-Trap** toggle in the **Power Controls** tab in Bluelake (see [Figure 11.15.1](#)).

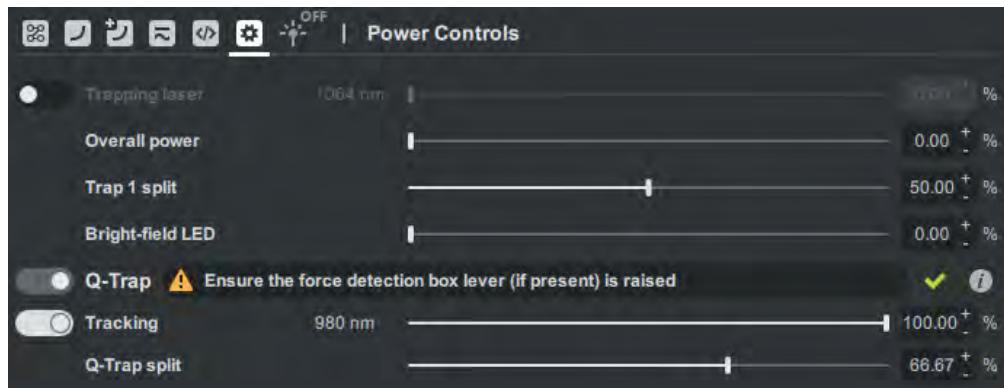


Figure 11.15.1: Figure: Switching to Q-Trap mode in the Power Controls panel.

2. On the force detection box, raise the lever to switch to the 980-nm tracking laser force detection on Trap #4, see the [Figure 11.15.2](#).



Figure 11.15.2: Figure: Q-Trap lever in the force detection box.

3. Close the lid and confirm (green checkmark) that you have raised the force detection box lever in Bluelake.
4. Switch on the **Tracking (980 nm)** laser and set it to 100% power.

Note: In the **Power Controls** tab, a **Q-Trap Split** ratio to 66.7% will distribute the laser power equally into Traps #1 & #2 and #3 & #4. This ratio is set automatically when you switch to Q-Trap mode. If you want to adjust the power ratio between Traps #1 & #2 and #3 & #4 to a different setting, you can use the **Q-Trap Split** slider. Additionally, you can use the **Trap 1 Split** slider to tune the ratio of laser power between Trap #1 and Trap #2. The ratio of laser power between Trap #3 and Trap #4 is always equal.

11.16 Temperature Control

If your C-Trap system is equipped with a Temperature Control feature, you can utilize it to control and stabilize the temperature of the experimental area.

Here is how to use the temperature control:

1. In Bluelake, you will find an additional interface for the temperature control (see [Figure 11.16.1](#)).



Figure 11.16.1: Temperature control settings in Bluelake.

2. Input the desired temperature value in the dedicated field.
3. Turn on the temperature control by sliding the toggle switch.
4. Orange icons will appear, indicating that the heaters are active and applying a certain percentage of power to achieve the desired temperature.
5. Allow the system some time to stabilize and reach the target temperature. Both the bottom (via objective) and top (via condenser) of the flow cell will be heated to maintain temperature uniformity.
6. To fine-tune the PID (Proportional-Integral-Derivative) parameters for temperature control, click on the ... icon to open a separate window (see [Figure 11.16.2](#)). In this window, you can manually adjust the PID parameters or initiate a self-tuning procedure by clicking the **Auto Tune** button.

To achieve optimal temperature and force stability during your experiments, LUMICKS recommends following these steps:

1. Set the **Overall Power** to a value greater than 5% (or the specific value required for your experiment).
2. Set the target temperature to the desired temperature for your experiment.
3. Turn on the temperature control by sliding the toggle switch.

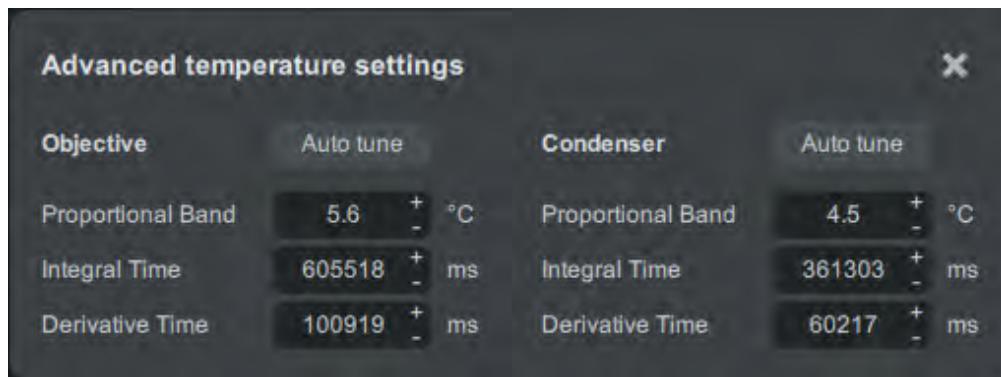


Figure 11.16.2: Advanced temperature control settings.

4. Allow the system to stabilize for at least 1 hour to ensure thermal equilibrium.
5. After capturing beads, wait for 3 minutes before starting the force calibration to ensure stable temperature conditions.

Note: If you are using Immersol (objective oil for the water objective) with the confocal system, be aware that it has a different refractive index for different wavelengths of visible light (dispersion). This can result in different focal planes for different excitation lasers, which may affect multi-color imaging.

11.17 Time line channels

The tables below gives a full overview of all possible data channels on the C-Trap. Which channels are available depends on your C-Trap configuration. The data channels under ATS, Focus-lock, IRM, Moon, WT and Z-finder are not all mentioned explicitly. Those channels names follow the same logic as data channels available for the Brightfield camera.

Table 11.17.1: General Timeline channels

Channel name	Unit	Description
Force HF - Force AB	pN	High Frequency force on trap A in the B direction recorded by the PSD
Force HF - Corrected Force AB	pN	High Frequency force with baseline subtracted
Force LF - Force AB	pN	Force on trap A in B direction downsampled to the BrightField Frequency
Force LF - Trap A	pN	Total force on Trap A downsampled to the BrightField Frequency $TotalForce^2 = Force_x^2 + Force_y^2$
Distance - Piezo Distance	µm	High (PSD) frequency Distance computed by subtracting the bead displacements from the Trap positions
Distance - Distance A	µm	Low frequency Distance computed from the template positions recorded by the Brightfield camera
Trap position - AB	µm	Position of Trap A along B axis
Nanostage position - A	µm	Position of the nanostage along axis A

continues on next page

Table 11.17.1 – continued from previous page

Channel name	Unit	Description
ATS - Power Correction	%	Correction signal to stabilize the laser power using the Overall Power waveplate in the Active Trap Stabilization (ATS) feature
ATS - Distance correction A	µm	Distance between the Trap 1 and Trap 2 laser spots which is being held constant in the Active Trap Stabilization (ATS) feature
Bead diameter - Template A	µm	Bead diameter of bead with template A
Bead position - Bead A B	µm	Position along axis B for bead with template number A
Diagnostics - Abaco temperature	C	Temerature of the Abaco laser
Diagnostics - Bright-field LED	%	Intensity Brightfield LED
Diagnostics - Bright-field tracking time	ms	Amount of time required to perform bead tracking on each frame
Diagnostics - Bright-field trigger	0/1	Brightfield camera frame acquisition trigger
Diagnostics - FD sync	0/1	TTL output line that is HIGH when the system is executing and FD curve LOW otherwise
Diagnostics - Microstage position A	mm	Position of the microstage along axis A
Diagnostics - Nanostage command A	µm	Nanostage axis A (XYZ) target position
Diagnostics - Pressure	bar	Current pressure
Diagnostics - Shutter A	0/1	Status of the shutter for Trap a (0 = open, 1 = closed)
Diagnostics - Trap 1 command A	µm	Trap 1 axis A (XY) target position
Diagnostics - Trap 1 encoder A	µm	Trap 1 axis A (XY) sensor position via digital readout
Diagnostics - Trap 1 power A	V	Voltage at the detector along axis A
Diagnostics - Trapping laser	%	The currently chosen intensity of the trapping laser
Diagnostics - Widefield trigger	0/1	Widefield camera frame acquisition trigger
Fluidics - Valve A	0/1	Status of valve A (0 = closed, 1 = open)
Focus lock - Position	µm	Z-coordinate of the focus lock at full bandwidth (500 Hz)
Focus lock - Filtered position	µm	Z-coordinate of the focus lock downsampled to 1 Hz
Focus lock - Correction	µm	Focus lock correction signal used for the feedback loop
Force feedback - Error	pN	Difference between current and target force
Force feedback - Command A	µm	PID output command to to selected device
Force feedback - Process Value	pN	Total force value used as input in the feedback loop
Power controls - Overall power	%	Overall power for the trapping laser

continues on next page

Table 11.17.1 – continued from previous page

Channel name	Unit	Description
Power controls - Q-Trap split	%	Controls distribution of power between Traps 1&2 and 3&4 on a Q-Trap
Power controls - Trap 1 split	%	Controls the power distribution between Trap 1 and 2
Temperature - Target	C	Target temperature at Condenser and Objective for systems with a Temperature Control feature
Temperature - Condenser/Objective	C	Measured temperature at Condenser/Objective
Tracking Mastch Score - Bead A	%	Matching score between template and bead number A

Table 11.17.2: Channels specific to C-Trap Dymo

Channel name	Unit	Description
Photon count- Red/Green/Blue	#	Photon counts recorded by APD
Confocal diagnostics - Red/Green/Blue/Violet shutter	0/1	Status of shutter for Red/Green/Blue/Violet excitation laser (0 = open, 1 = close)
Confocal diagnostics - Mirror set position A	μm	Set position for mirror excitation laser along axis A
Confocal diagnostics - Mirror actual position A	μm	Actual position for mirror excitation laser along axis A
Confocal diagnostics - Pixel clock	0/1	Has value 1 when new Pixel starts, 0 otherwise
Confocal diagnostics - Line clock	0/1	Has value 1 when new Line starts, 0 otherwise
Confocal diagnostics - Frame clock	0/1	Has value 1 when new Frame starts, 0 otherwise
Confocal diagnostics - Excitation Laser Red/Green/Blue/Violet	%	Intensity of the respective excitation laser
Confocal diagnostics - Depletion Laser STED	%	Intensity of the depletion laser for STED
Info wave - Info wave	0/1	Contains instructions on how to reconstruct a scan from the photon count channel

11.18 Shutting down the system (end of day)

1. In Bluelake, set the **Trapping Laser** power to zero.
2. Switch off the photon counting module in the electronics rack.
3. Press **Raise** in the **Condenser Control** menu to raise the condenser.
4. Retract the objective downwards by rotating the **objective knob** counter-clockwise.
5. Clean the microscope objective lens with a lens cleaning tissue.



Figure 11.17.1: The list of data channels in Bluelake.

6. Clean the condenser lens with a different lens cleaning tissue.
7. Clean any residual water on the bottom of the flow cell and oil on top of the flow cell.
8. Close the lid of the C-Trap.
9. Quit Bluelake by pressing the **X** button in the upper right corner of the window and select **Yes, put my system in a safe state** (see [Figure 11.18.1](#)).
10. Turn the key on the Trapping laser in the electronics rack counter-clockwise to turn it off.

Note: Only when you intend to shut down the C-Trap for a long period or when performing a power-cycle, turn off the main power switch located on the front panel of the electronics rack.

11.19 Shutting down the system for a short break

1. In Bluelake, set the **Trapping Laser** power to zero.
2. Switch off the photon counting module in the electronics rack.
3. Press **Raise** in the **Condenser Control** menu to raise the condenser.
4. Retract the objective downwards by rotating the **objective knob** counter-clockwise.
5. Clean the microscope objective lens with a lens cleaning tissue.

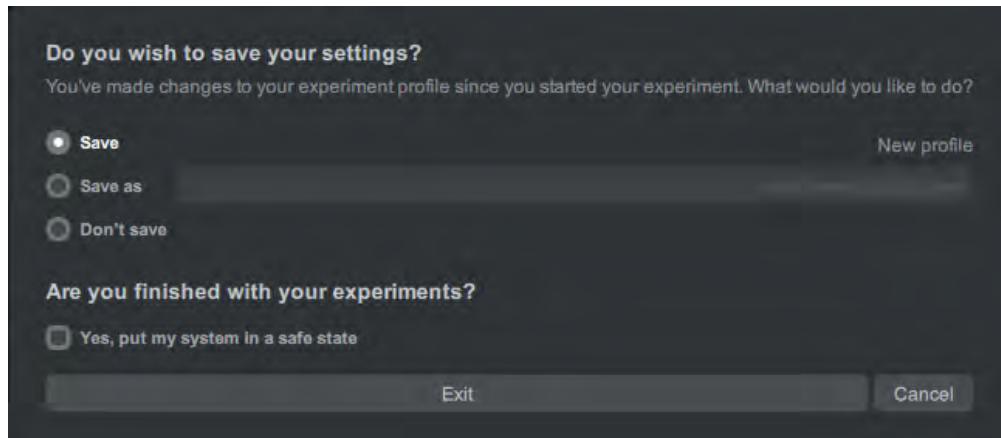


Figure 11.18.1: Figure: Shutdown confirmation window.

6. Clean the condenser lens with a different lens cleaning tissue.

Note: Make sure you clean the objective lens if you use water, to avoid damage due to corrosion.

7. Clean the residual water on the bottom of the flow cell.
8. Close the lid.

11.20 Waste disposal

The safe and responsible disposal of liquid waste, including cleaning reagents, microplastics such as polystyrene spheres, DNA and protein samples in solution, is a critical aspect of operating the C-Trap. Be sure to familiarize yourself with the section on waste disposal of the Safety Data Sheet (SDS) of reagents and compounds used. Before you proceed with any waste disposal, make sure you are equipped with appropriate Personal Protective Equipment (PPE). Always be aware of the chemical and biological hazards associated with the waste you are handling, and familiarize yourself with both local and national waste disposal regulations.

To start the disposal process, it is important to segregate the waste based on its chemical and biological nature. Never mix different types of waste together, as this could result in hazardous reactions or complicate the disposal process. Use containers that are specifically labeled "Bio-hazardous Waste" if appropriate. Make sure these containers are sealed and stored in designated areas until they are ready for disposal. Your facility may have its own waste management procedures, so consult those localized guidelines for additional instructions on how to handle biohazardous waste. For the disposal process, rely on authorized waste management services to collect the waste material.

12 Appendix

12.1 U-Flux - Automatic fluidics setup

Warning: Valve fatigue or incorrect connection of the tubing can result in leakage. To make sure the C-Trap housing or your set-up is not affected in this unlikely case, LUMICKS recommends using the tooling provided and following these instructions.

Note: If tubing is intended to be replaced, it is recommended to use a mat under the U-Flux assembly to avoid any splash on the instrument.

1. Cut 2 pieces of tubing to the desired length to connect the pressure box with the Automatic fluidics and the latter with the Flow Cell (always use an appropriate tubing cutter to ensure a good connection). Unscrew the fittings and guide the tubing through, connecting the corresponding ferrule from the other side (see [Figure 12.1.1](#) and [Figure 12.1.2](#)).

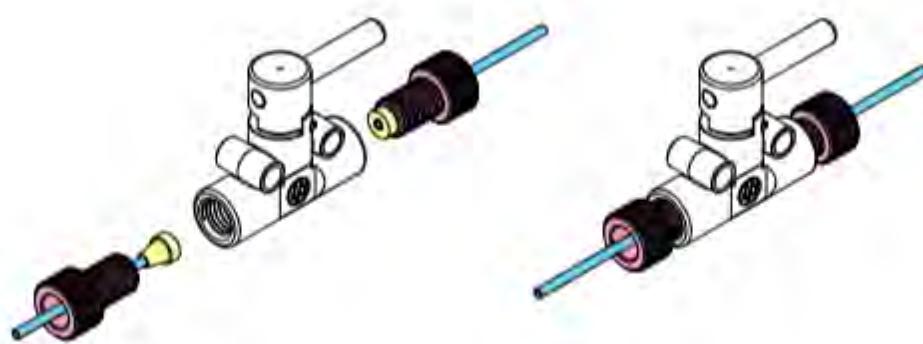


Figure 12.1.1: Figure: Exploded view of the valves.

2. Screw the fittings into the valve by hand (see [Figure 12.1.3](#)).
3. Once the fittings are screwed in by hand, and in order to assure a good seal, use the provided **torque screwdriver + adapter** (see [Figure 12.1.4](#)) to achieve the desire and secure torque (**0.45 Nm**).

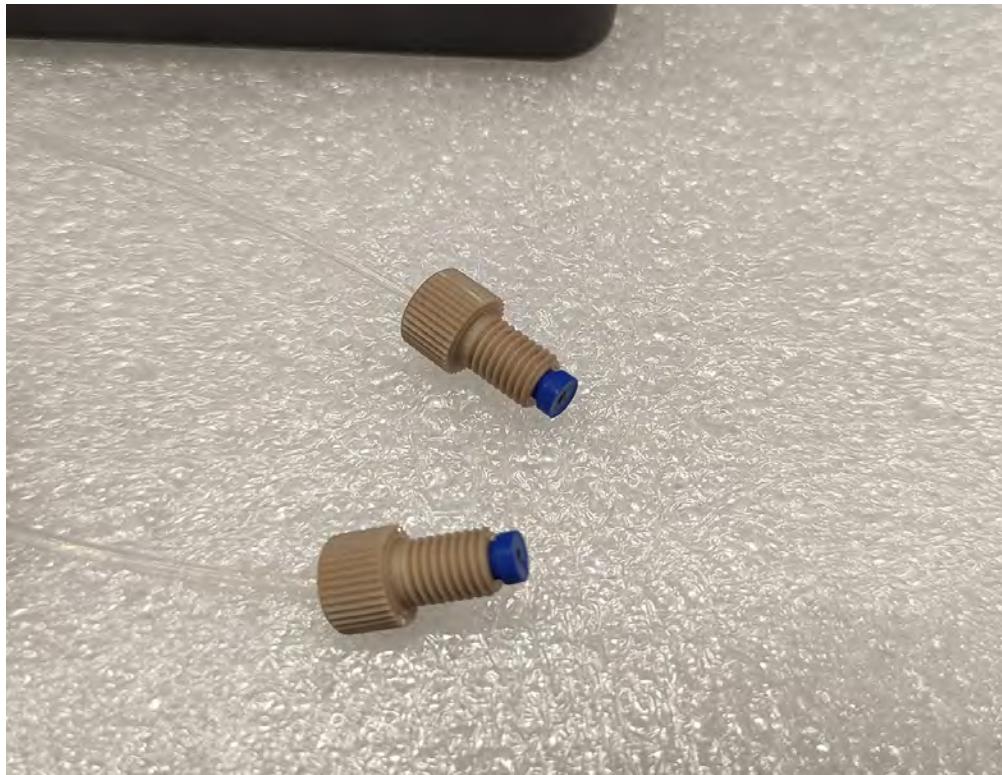


Figure 12.1.2: Figure: Tubing with ferrules.

4. Make sure the tooling is set with the required torque of **0.45 Nm**. If the setting is correct, the value “0.45” should be readable and the arrow must point the line bellow (see [Figure 12.1.5](#)).
5. Torque setting is easily achieved by simply pushing down and turning the adjustment ring. With each 180° turn the torque value will be changed and the set up ring will engage audibly. Clockwise direction increases and anti-clockwise decreases the torque value (see [Figure 12.1.6](#)).
6. To use the tool, make sure that the **tubing** is accommodated through the opening of the adapter and that the **adapter goes all the way in** (see [Figure 12.1.7](#)).
7. When the right torque (**0.45 Nm**) is achieved, **a click will be heard** and no longer torque will be applied. It is recommended to **tighten twice each fitting** (if the right torque was set, the second should be heard soon after).
8. Proceed to **tighten all the valves** with the same procedure.
9. Once the fittings are properly installed at the valves, proceed to connect the other ends at both the **Pressure Box** (see [Figure 12.1.8](#)) and **Flow Cell** (see [Figure 12.1.9](#)).

Note: Please make sure to make a proper connection between the valves and the tubing, as this is the main reason for leakage.

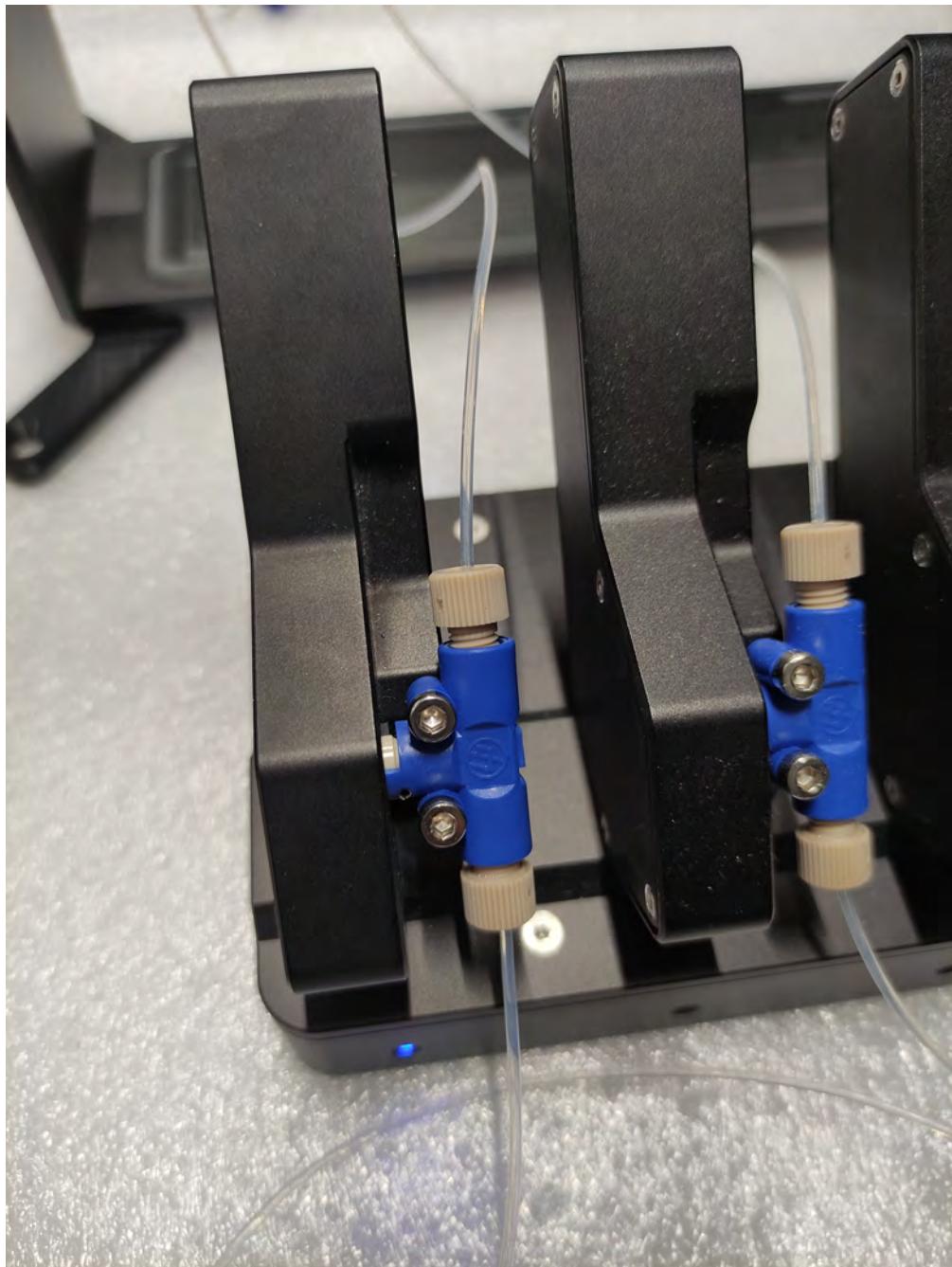


Figure 12.1.3: Figure: Screwing the fittings by hand at the valve.



Figure 12.1.4: Figure: Torque screwdriver with the valve adapter.



Figure 12.1.5: Figure: Torque value setting.

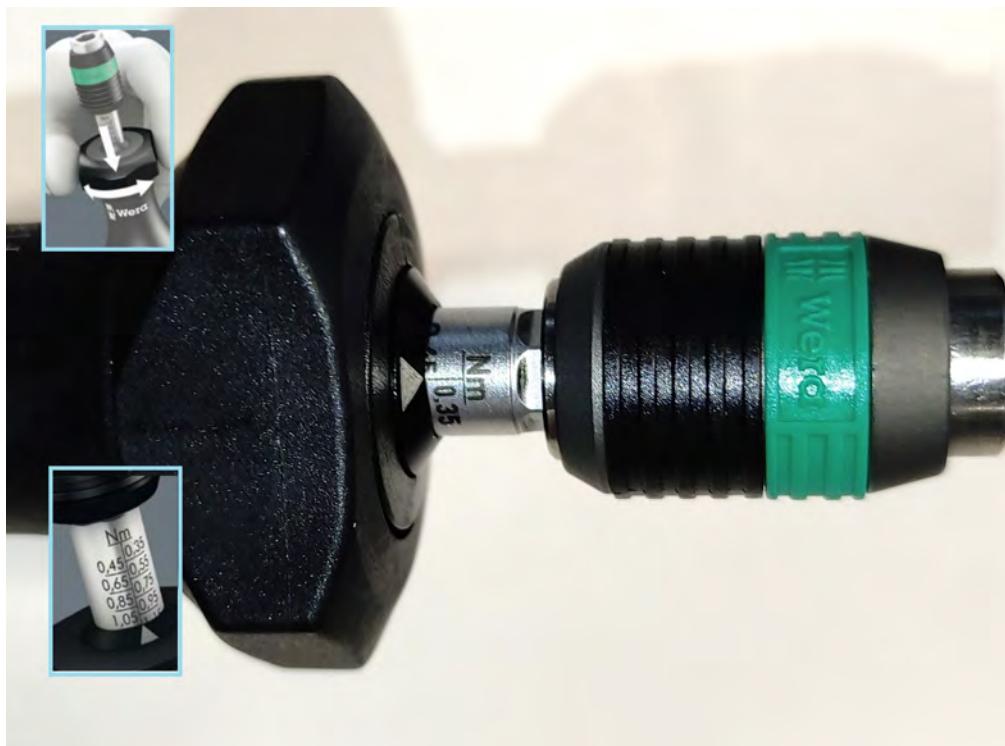


Figure 12.1.6: Figure: How to set correct torque in the screwdriver.

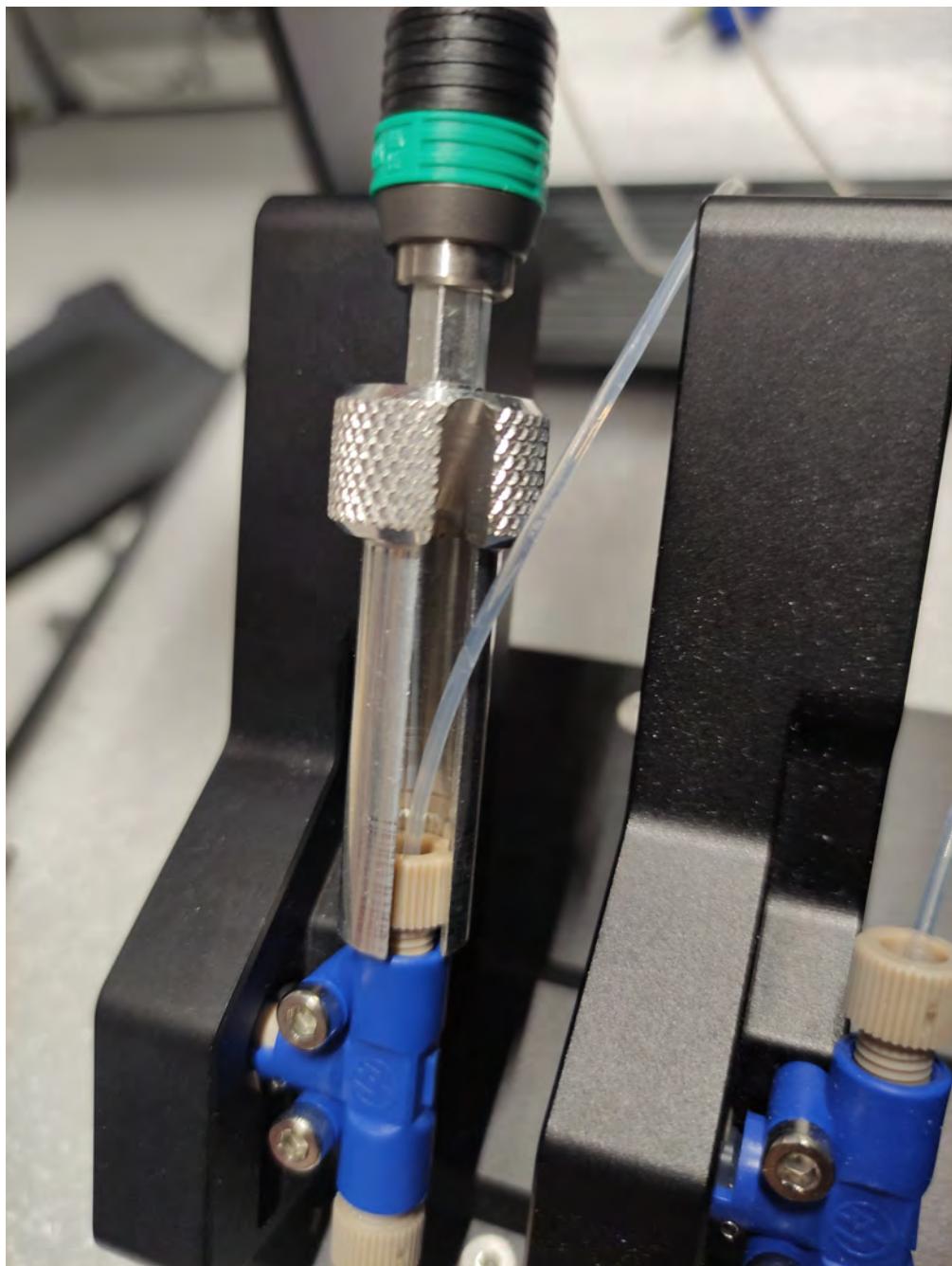


Figure 12.1.7: Figure: How to place the screwdriver into the fittings.

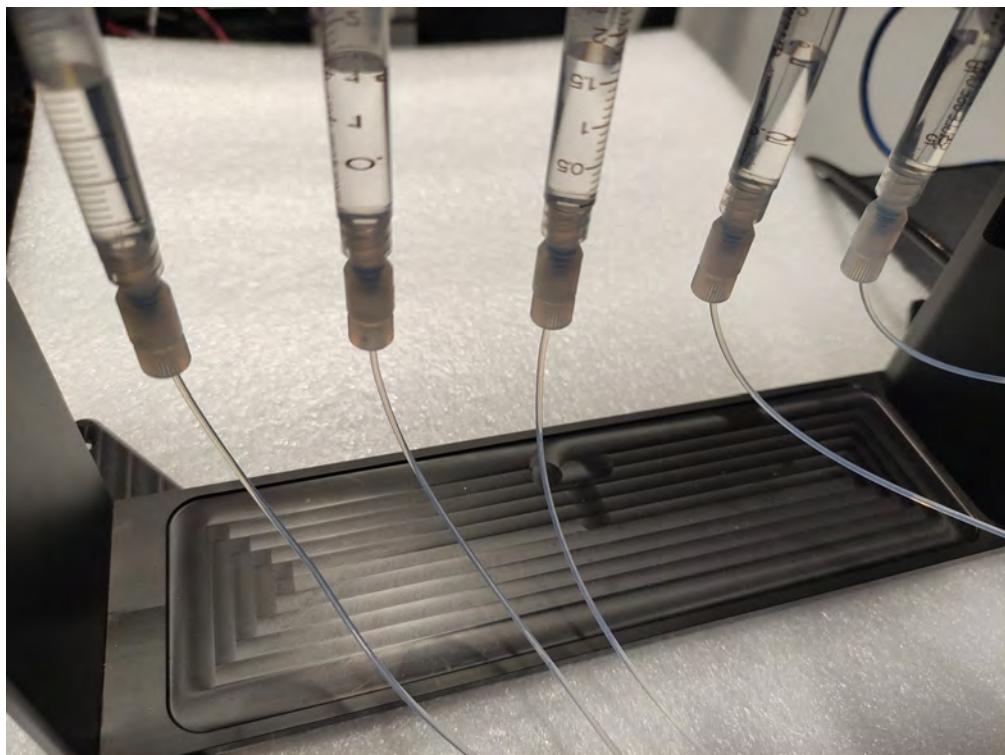


Figure 12.1.8: Figure: Connection at the Pressure Box.

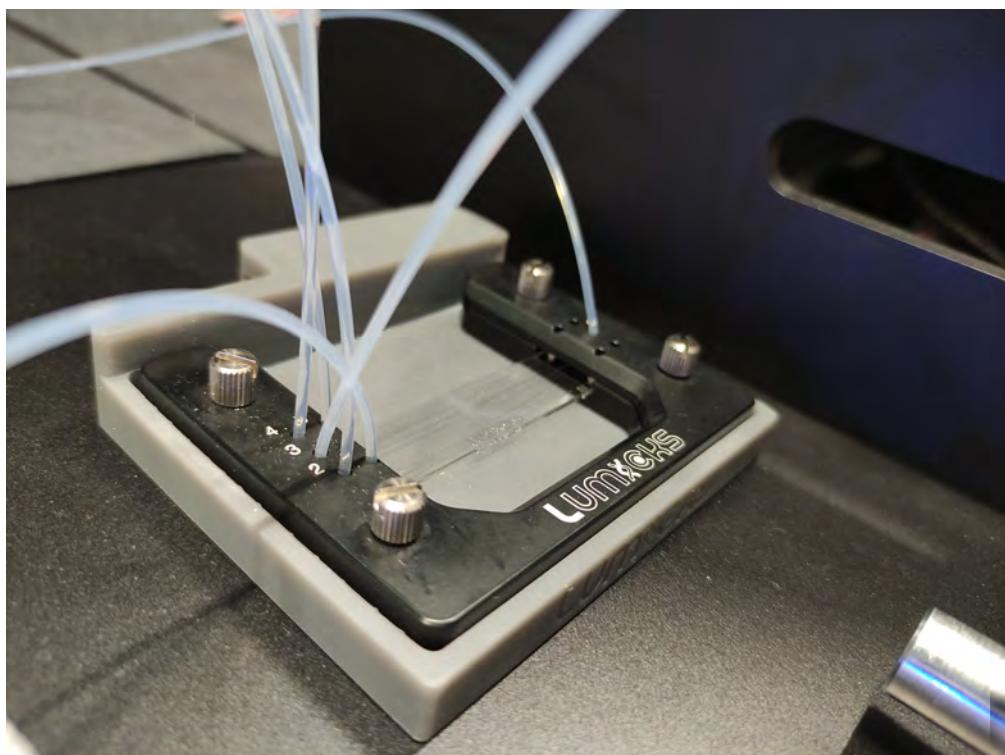


Figure 12.1.9: Figure: Connection at the Flow Cell.

12.2 Exchanging the flowcell tubing

The following shows step by step how the tubing is microfluidics tubing is removed from the flowcell and reinserted.

1. Move the flowcell mount from the slide adapter to the front such that you can handle it.
Make sure to have sufficient long tubing.
2. Release all four thumbscrews on the flowcell mount (see [Figure 12.2.1](#)).

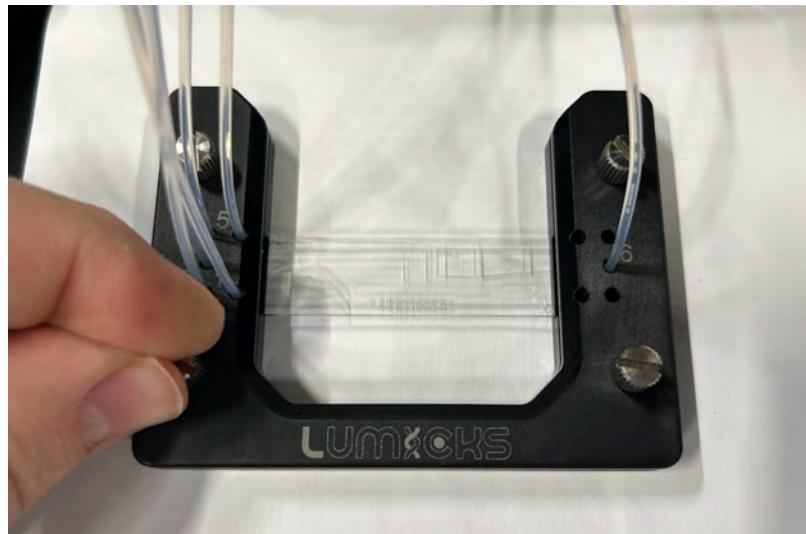


Figure 12.2.1: Figure: Release all thumbscrews on the flowcell mount.

3. Gently pull out the tubing, one by one (see [Figure 12.2.2](#)).

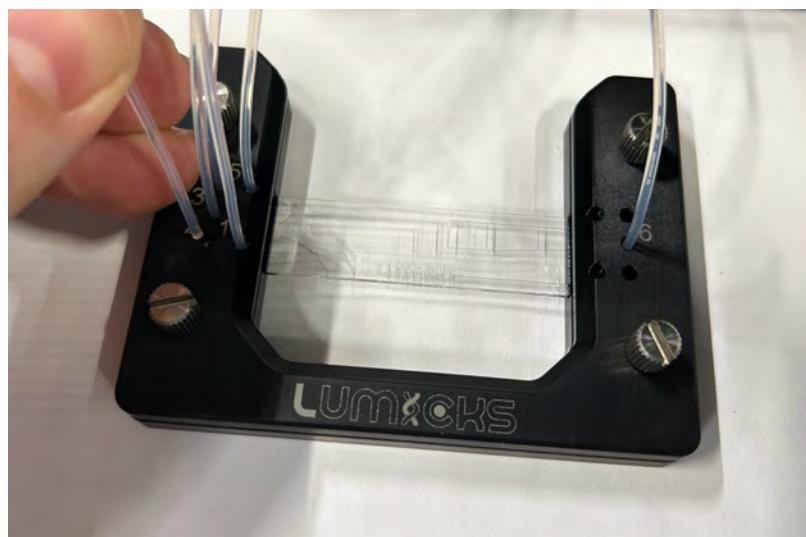


Figure 12.2.2: Figure: Gently pull out the tubing one by one.

4. To insert new tubing into the flowcell, push the tubing straight into the ferrule through the flowcell mount throughhole (see [Figure 12.2.3](#)).

Note: When adjusting the length to replace tubing, only use a tube cutting tool to ensure well-cut endfaces of the tubing and avoid squishing. This makes sure to avoid any leakages at the ferrules or reduced tubing diameters which can affect the performance of the microfluidics.



Figure 12.2.3: Figure: Push the tubing straight into the ferrule through the flowcell mount throughhole.

5. Tighten all four thumbscrews on the flowcell mount.

12.3 Aligning a sample using the tip-tilt sample holder

The tip-tilt sample holder allows for precise alignment of a custom sample with the imaging and trapping focal planes. Follow these step-by-step instructions to perform the alignment:

1. Mount the custom sample in the tip-tilt sample holder using the clamps (see [Figure 12.3.1](#)).
2. Place the tip/tilt sample holder with the custom sample in the sample area of the C-Trap.
3. Familiarize yourself with the layout of the sample channel and position the microscope objective, allowing 1 mm movement in both horizontal and vertical directions while remaining within the sample area.
4. Close the lid.
5. Press the red **RESET** button on the trapping laser and then turn it clockwise until the knob releases towards you. Once released, press the green **START** button, which should illuminate to indicate the laser is on.
6. Use the z-finder to focus on the measurement surface area of the custom sample. It is advisable to use low laser powers to maintain visibility of the reflections.



Figure 12.3.1: Figure: Custom sample holder.

Note: When using the TIRF objective, the first surface reflection is very weak due to the index-matching of the immersion medium and glass.

7. The next steps will guide you in adjusting the tilt in the X and Y directions:
 - a. With the measurement surface in focus on the z-finder camera, move the microstage 1,000 μm to the right and then 1,000 μm back to the original position. Observe any change in focus. If the focus remains unchanged, the sample is level in the X direction. If there is a change, proceed to the next steps to adjust the sample tilt in the X direction.

Note: Ensure that the microscope objective continues to image your sample medium during these movements to maintain focus. If needed, smaller moves can be made if the sample does not allow for a 1,000 μm adjustment, although this may result in less precise tilt adjustment.

- b. Turn off the trapping laser and open the lid. Rotate the bottom-right knob on the tip/tilt sample holder about half a turn in a specific direction. Close the lid and turn on the trapping laser (step 5). Refocus the z-finder camera on the second surface. Repeat the movement described in step 7a and observe whether the change in focus has improved or worsened. If it has worsened, reverse the adjustment direction at the start of this step by rotating the bottom-right knob.
 - c. Repeat step 7b, taking progressively smaller adjustments with the knob as the changes in focus become smaller. Be cautious not to make large adjustments that could cause overshooting in the wrong direction. Continue until the focus no longer noticeably changes. This indicates that the sample is now level in the X direction.
 - d. With the measurement surface in focus on the z-finder camera, move the microstage 1,000 μm upward and then 1,000 μm downward to the original position. Note any change in focus. If the focus remains unchanged, the sample is level in the Y direction. If there is a change, proceed to the next steps to adjust the sample tilt in the Y direction.
 - e. Turn off the trapping laser and open the lid. Rotate the top-left knob on the tip/tilt sample

stage about half a turn in a specific direction. Close the lid and turn on the trapping laser (step 5). Refocus the z-finder camera on the second surface. Repeat the movement described in step 7d and observe whether the change in focus has improved or worsened. If it has worsened, reverse the adjustment direction at the start of this step by rotating the top-left knob.

- f. Repeat step 7e, making smaller adjustments with the knob as the changes in focus become smaller. Be cautious not to make large adjustments that could cause overshooting in the wrong direction. Continue until the focus no longer noticeably changes. This indicates that the sample is now level in the Y direction.
 - g. Conclude the procedure by verifying that the sample is level in the X direction again, as adjustments in the Y direction can affect the other axis.
8. Now the sample is level.

12.4 Bridge PC and Controller PC

The C-Trap system includes two computer systems: the **Controller PC** and the **Bridge PC**. Here are important guidelines regarding their usage:

1. Controller PC:

- It is directly connected to the C-Trap and is used for experiment control.
- The Controller PC has been configured by LUMICKS and should not be modified.
- It is not connected to the network or the internet for robustness and security reasons.
- The Controller PC is solely dedicated to operating the C-Trap and experiment control.

2. Bridge PC:

- The Bridge PC acts as a bridge between the Controller PC and the local network.
- It can be used to access the internet based on your local network policy.
- The Bridge PC allows communication between the Controller PC and the network.

Note: It is critical to maintain the configuration of the Controller PC without any modifications and to keep it disconnected from the network or the internet. Do not install additional software and do not perform Windows Updates.

Please note that the Bridge PC can be customized and adjusted according to your IT department's security standards. However, it is crucial to maintain the unchanged network connection configuration with the Controller PC and avoid uninstalling any original software provided.

Note: LUMICKS cannot be held responsible for any security issues that may arise from customization of the original configuration of the Controller PC and Bridge PC.

Please be aware that the Controller PC and the Bridge PC of a C-Trap system are configured solely for operation and temporary data storage purposes.

LUMICKS strongly advises storing measurement data on a secure external location, such as a data server or network drive that undergoes regular backups. LUMICKS cannot be held liable for any data loss resulting from a computer failure.