Ampha™ Z32

Impedance Flow Cytometer

Instrument User Guide

Release 2.1.6

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Contents

1 Introduction

The Ampha Z32 is the newest generation of the Amphasys Impedance Flow Cytometers. The core of the cytometer is a microfluidic chip, capable of measuring electrical properties of virtually any kind of cells (yeasts, plant, pollen, bacteria, animal and human cells). The technology does not require the use of specific labels. Thus, the sample preparation is very easy and quick. The device can measure changes in cell size, membrane capacitance and cytoplasmic conductivity, parameters whose alterations characterize many cellular processes. It is best suited for routine, pre-diagnostic and quality control analyses. In addition, it covers many classical research applications, as for example apoptosis, cell differentiation or development and ploidy analyses.

1.1 About the User Guide

This User Guide provides detailed information about the Ampha Z32 Impedance Flow Cytometer specifications, setup, measurements, autosampler operation, maintenance, troubleshooting and service.

The Ampha Z32 system is operated using the software AmphaSoft 2.0. AmphaSoft 2.0 runs on a Windows-based laptop PC. Please refer to the *AmphaSoft 2.0 User Guide* for more information.

Further details, answers to frequently asked questions, tutorial movies and downloads are available at https://amphasys.com/ampha-z32-pollen-analyzer/#tutorials.

1.2 Delivery and Inspection

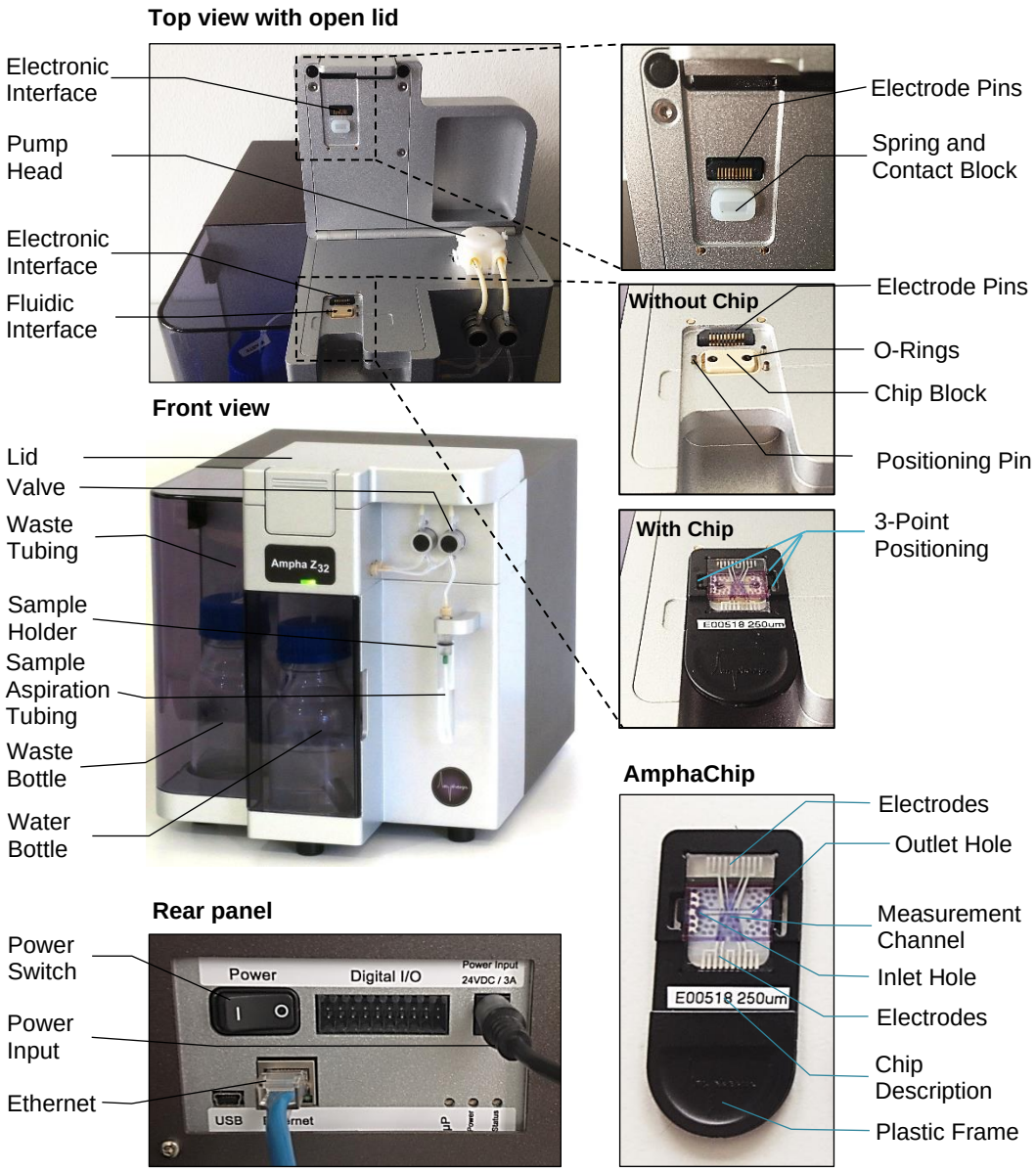
Carefully inspect all package boxes upon receipt of the cytometer. If there are any signs of mishandling or damage, file a claim with the carrier immediately. If the shipment is separately insured, file a claim with the insurer.

1.3 Ampha Z32 Specifications

Impedance Chips	A chip: 15 µm x 15 µm
	B chip: 30 µm x 30 µm
	C chip: 50 µm x 50 µm
	F chip: 80 µm x 80 µm
Channel dimensions	D chip: 120 µm x 120 µm
	E chip: 250 µm x 250 µm
	G chip: 400 µm x 300 µm
	Other chip types on request depending on application
Impedance Measurement	
Frequency range	100 kHz – 30 MHz
Frequency selection	Up to 4 different frequencies simultaneously
Analysis Range	
Sample volume	50 – 4000 µl
Concentration range	1 x 10^3 to 1 x 10^7 cells /ml, depending on chip type
Particle size	1 - 250 µm
Fluidics	
Sample flow rate	5 – 4000 µl/min, depending on chip
Pump	Peristaltic pump with disposable pump head
Labware compatibility	5 ml polystyrene/polypropylene round-bottom tubes
	(Falcon® PP 352002/PS 352003/ Sarstedt PP 55.1579)
PC Operating System	Windows 7 or 10 Pro
Software	AmphaSoft 2.0
Dimensions	255 x 275 x 353 mm (W x D x H)
Weight	8.4 kg
Operating Environment	
Temperature	16 – 32 °C
Humidity	10 % – 90 % relative non-condensing
Power	24V DC ± 10 %, max. 3 A, < 90 W

2 Ampha Z32 System and Parts

The Ampha Z32 Impedance Flow Cytometer is a high-tech bioanalytical instrument. It comprises sophisticated electronic, fluidic and software components. The cytometer is operated from an external PC via USB-to-Ethernet connection (or Ethernet only). The pictures below show the main Ampha Z32 components.



2.1 Fluidics

The purpose of the fluidic system is the transport of the sample to the chip. The transport process is driven by a peristaltic pump. This pump aspirates sample via the sample aspiration tubing. Samples can be delivered to the instrument in several ways. The most convenient option is to use 5 ml round-bottom tubes, which can be directly plugged to the sample adapter. However, the flexible sample aspiration tubing can also be inserted into microcentrifuge tubes, Falcon tubes, well-plates and other standard sample containers. After being aspirated through the sample aspiration tubing and the peristaltic pump, the sample is transported towards the chip. Two O-rings form a watertight contact between the system tubing and the AmphaChip. The sample enters the chip through the inlet hole, passes the measurement unit, and leaves the chip through the outlet hole. A flow sensor quantifies the amount of liquid that passes the chip. Finally, the sample reaches the waste bottle via the waste tubing.

The Ampha Z32 incorporates a second fluidic system with the purpose of cleaning the first fluidic system. This system aspirates deionized water from the water bottle and flushes the lines in both directions towards the sample aspiration tubing and the waste tubing.

In order to switch between the measuring and rinsing fluidic processes, two pinch valves selectively activate only one fluidic path at a time.

More details of fluidic pathways are explained in the Maintenance section (6.1).

2.2 Electronics

The Ampha Z32 is equipped with several electronic boards and other electronic components. Data transmission from and to the PC happens via Ethernet (with or without USB adapter). The electronic interface to the AmphaChips occurs via rows of electrode spring contacts that are brought into direct contact with the electrodes that are patterned on the AmphaChips. One row of electrode pins is located in the instrument lid, and the other row is integrated into the chip holder. These electrodes are used for the generation of the electric field and the reception of the impedance signals.

2.3 Software

The Ampha Z32 software system consists of instrument software and PC software. The PC software is called AmphaSoft 2.0 and is used to operate the instrument, to plan experiments and to perform data analysis and reporting. More information about AmphaSoft 2.0 is available in the *AmphaSoft 2.0 User Guide*.

3 Hazards and Precautions

This handbook contains information and warnings that must be followed by the user to ensure safe operation of the instrument and to maintain the instrument in a safe condition. Possible hazards that could harm the user or result in damage to the instrument are clearly stated at the appropriate places throughout this handbook.

Before using the instrument, it is essential to read this handbook carefully and to pay particular attention to any advice it contains concerning hazards that may arise from use of the instrument. Advice given in this handbook is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country. Carry out the maintenance regularly in accordance with the operating instructions. Amphasys will charge for repairs that prove to be required due to incorrect maintenance.

3.1 Electrical Safety

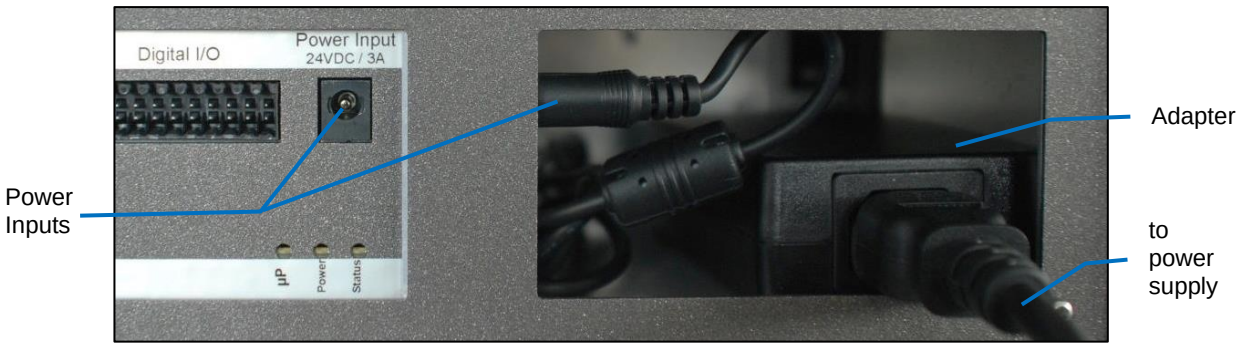
To ensure satisfactory and safe operation of the instrument, it is essential that the neutral line power cord is connected to true electrical earth (ground).

When working with the instrument:

- Make sure the line power cord is connected to a line power outlet that has a protective conductor (earth/ ground).
- Do not attempt to make any internal adjustments or replacements.
- Do not operate the instrument with any covers or parts removed.
- If water or reagent has spilled inside the instrument, switch off the instrument and disconnect it from the line power supply. Contact Amphasys AG or the authorized distributor.
- Servicing should be carried out only by Amphasys AG or the authorized distributor.
- If the instrument becomes electrically unsafe* for use, make the instrument inoperative and secure it against unauthorized or unintentional operation. Contact Amphasys AG or the authorized distributor.

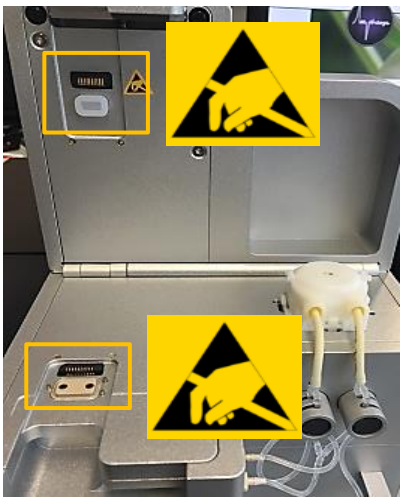
*) The instrument is likely to be electrically unsafe when:

- it shows visible damage,
- the line power cord shows signs of damage,
- it has been stored under unfavorable conditions for a prolonged period, or
- it has been subjected to severe transport stresses.



WARNING! Electrical Hazard The power supply is connected either at the rear of the instrument, or inside the instrument housing. The instrument is supplied with a 90 W industrial adaptor power supply, which provides output voltages of max. 24 V. Therefore, no lethal voltages are supplied inside the instrument. However, any interruption of the protective conductor (earth/ground lead) of the AC power supply cable or damage of the cable insulation is likely to make the instrument dangerous. Intentional interruption is prohibited. Use only the supplied power supply! Any other power supply might lead to hardware damage.

Attention! Don't touch the gold contact pins. You might be electrostatically charged. Discharging via the gold contacts can damage the electronics. In addition, contamination of the electrode surfaces can lead to electrode damage and decrease the quality of the instrument – chip interface.



3.2 Biological Safety

WARNING! Samples containing infectious agents - If you use infectious agents with this instrument, handle such samples with the greatest of care and in accordance with the required safety regulations. The responsible body (e.g. laboratory manager) must take the necessary precautions to ensure that the surrounding work place is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Materials Safety Data Sheets (MSDS) or other regulatory documents. Disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

WARNING! Waste disposal - Waste containers may contain hazardous chemicals or infectious agents from the process. Such wastes must be collected and disposed properly in accordance with the local safety regulations. Refer to your local safety regulations for proper disposal procedures.

3.3 Chemicals

WARNING! Hazardous chemicals - Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run (e.g. system cleaning solution). Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding work place is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Materials Safety Data Sheets (MSDS) or other regulatory documents. Disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

4 Setting up the Ampha Z32 Impedance Flow Cytometer

4.1 Installation and Training

Amphasys provides on-site instrument installation and Ampha Z32 training. Please contact your sales representative for detailed information.

4.2 PC

The Ampha Z32 Impedance Flow Cytometer is controlled with a Windows-based laptop PC. Owing to performance specifications and initial configurations, Amphasys recommends the purchase of a suitable laptop together with the instrument via Amphasys. The *AmphaSoft 2.0 User Guide* provides the required PC specifications.

4.3 Software and Licensing

The software used to operate the Ampha Z32 instrument is AmphaSoft 2.0. AmphaSoft 2.0 also provides data analysis and reporting tools. AmphaSoft 2.0 and any updates can be downloaded from the Amphasys website (https://amphasys.com/amphasys-downloads/#software-downloads). The name AmphaSoft 2.0 includes all releases of AmphaSoft 2.0. On the website, you can also register for E-mail notifications about software updates. More information about AmphaSoft 2.0 is available in the *AmphaSoft 2.0 User Guide*.

The use of AmphaSoft 2.0 requires a license. An instrument can be equipped with a standard license, which allows the use of AmphaSoft 2.0 in online mode (i.e. when the instrument is actively connected to the PC with the license). Alternatively, licenses that also allow offline operation (for experiment setup, data analysis and reporting) are called professional licenses and are available from Amphasys. Please contact your sales representative for more information.

4.4 Ampha Z32 Instrument

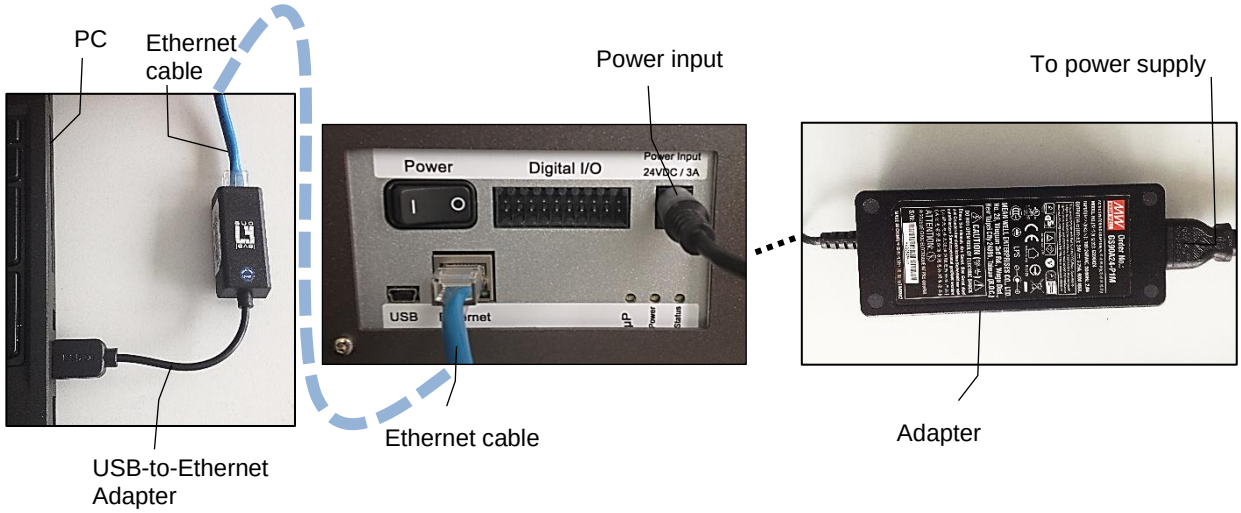
The instrument must be connected to an AC power outlet with 110-240V AC, 50/60 Hz. The power lines to the equipment should be voltage regulated and surge protected. Connect the instrument via Ethernet port to the laptop using either an Ethernet cable or and Ethernet cable with an Ethernet-to-USB adapter. Note that the occupation of the Ethernet port by the Ampha Z32 can impede the wireless internet connection of the PC. This reduces the possibilities of remote supporting. In order to ensure constant wireless internet accessibility, Amphasys recommends the use of an Ethernet–to-USB adapter.

- **Ethernet:** An Ethernet cable is used to connect the Ethernet port of the instrument with the Ethernet port of the PC.
- **Ethernet-to-USB (recommended):** This connection involves an Ethernet cable connected to the Ethernet port of the Ampha Z32, which is connected to a USB port of the laptop via an Ethernet-to-USB adapter. This configuration ensures constant wireless internet accessibility for remote support.

Note The Ethernet and Ethernet-to-USB setups can be used interchangeably. However, prior to switching, the ports must be configured accordingly. Amphasys does not recommend changing the configurations back and forth.

Instructions for configuring and changing Ethernet ports are provided in section *4.5 Configuration of ports for Ethernet or Ethernet-to-USB configurations*.

Ampha Z32 Setup using an Ethernet-to-USB connection



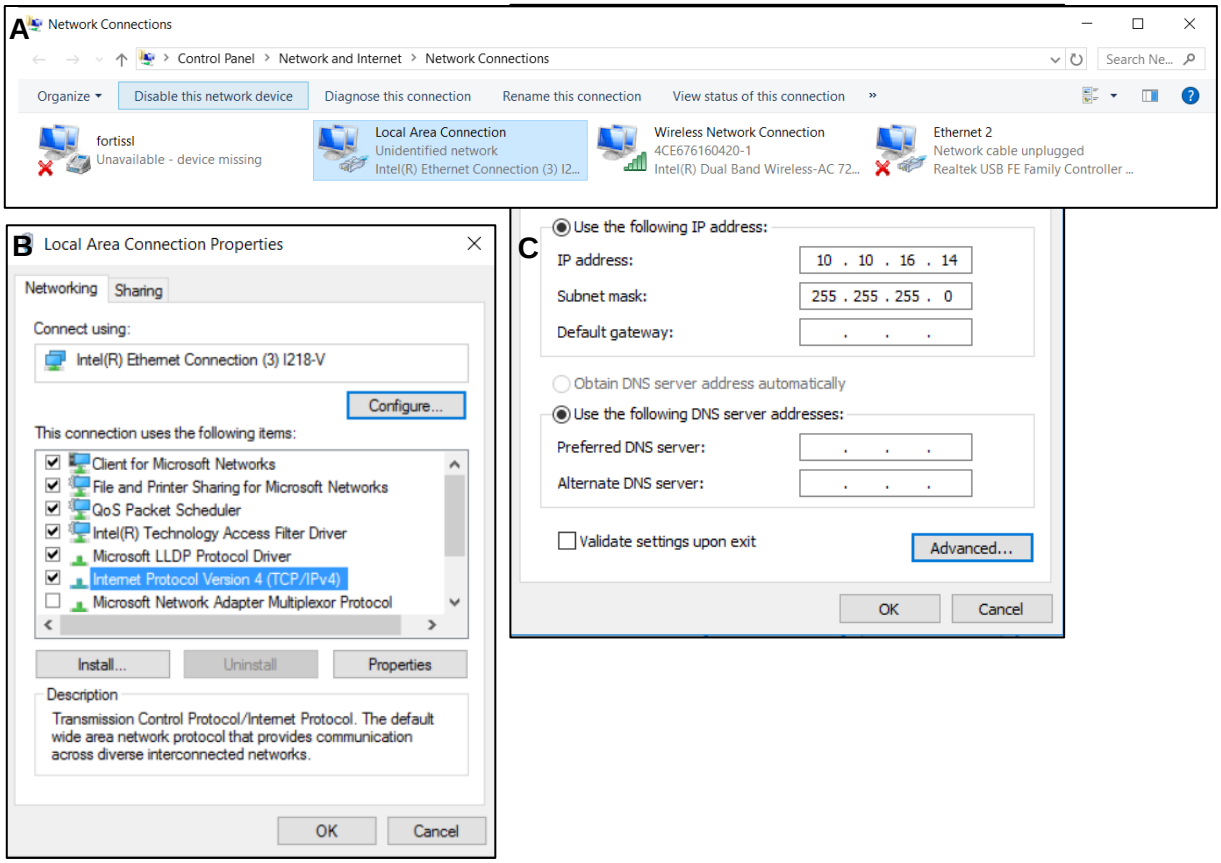
Attention! Always unplug all cables before moving the instrument or laptop.

To protect operating personnel, the National Electrical Manufacturers Association (NEMA) recommends that the instrument is grounded correctly. The instrument is equipped with a 3 conductor AC power cord. When connected to an appropriate AC power outlet, ground the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no grounding connection.

4.5 Configuration of ports for Ethernet or Ethernet-to-USB configurations

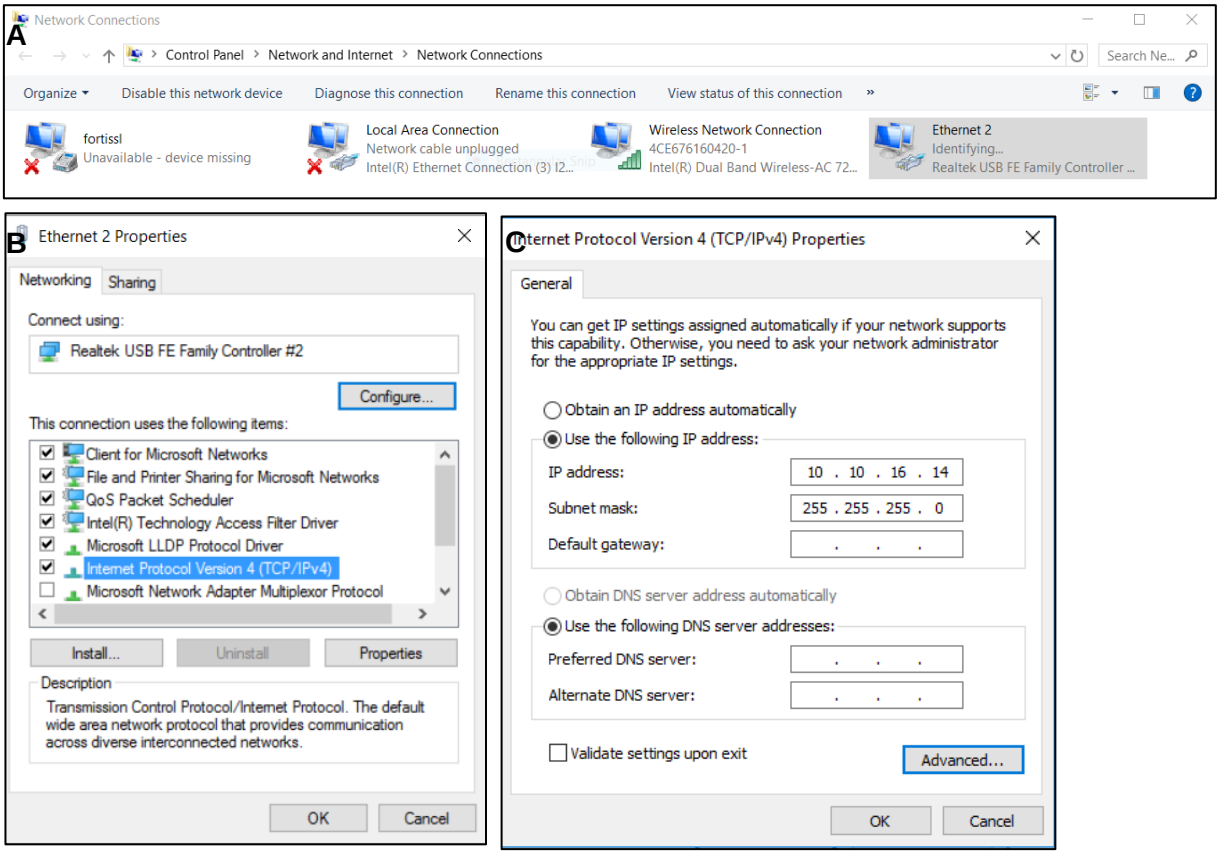
4.5.1 Initial setup of Ethernet port for Ethernet connection

- Connect your instrument to the laptop using the ethernet cable
- Switch the instrument on
- Open the *Network Connections* panel of your PC (*Settings* > *Network & Internet* > *Change Adapter Options*)
- Right-click on the Ethernet port that will be used to connect to the instrument and select *Properties*. Note: To find the correct port, you can unplug and plug the ethernet cable to see which connection is activated upon switching on the instrument (Fig. A)
- From the list, select *Internet Protocol Version 4 (TCP/IPv4)* > *Properties* (Fig. B).
- Change the dialog exactly as shown in the Figure C.
- Confirm with *OK*, close the previous dialog with *Close*



4.5.2 Initial setup of USB port for Ethernet-to-USB connection

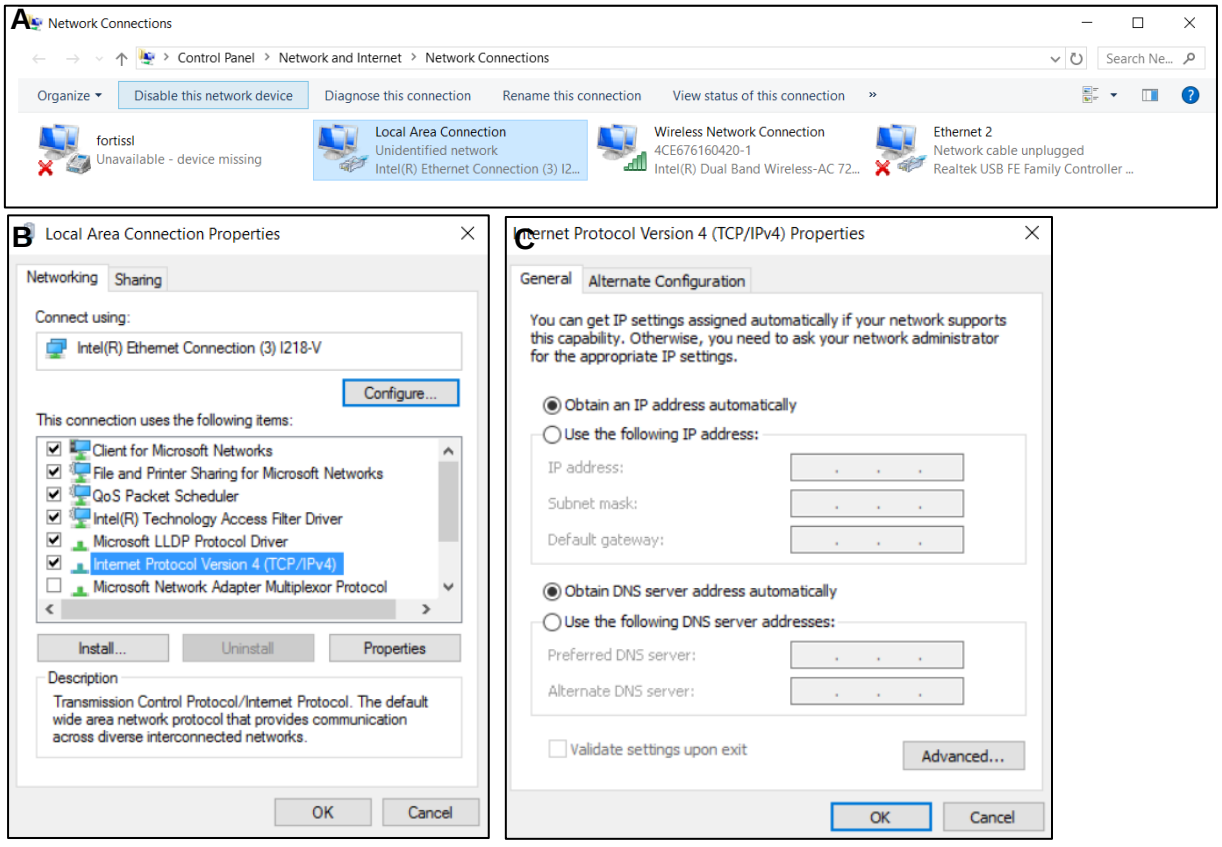
- Connect your instrument to the laptop using the ethernet cable and Ethernet-to-USB adapter
- Switch the instrument on
- Open the *Network Connections* panel of your PC (*Settings* > *Network & Internet* > *Change Adapter Options*)
- Right-click on the Ethernet port that will be used to connect to the instrument and select *Properties*. Note: To find the correct port, you can unplug and plug the Ethernet-to-USB adapter to see which connection is activated upon switching on the instrument (Fig. A). The correct port has the description "*Realtek USB FE Family Controller*"
- From the list, select *Internet Protocol Version 4 (TCP/IPv4)* > *Properties* (Fig. B).
- Change the dialog exactly as shown in Figure C.
- Confirm with *OK,* close the dialog with *Close.*



4.5.3 Change from Ethernet to Ethernet-to-USB

Follow this procedure if you used the Ethernet-only connection and want to change to Ethernet with USB adapter.

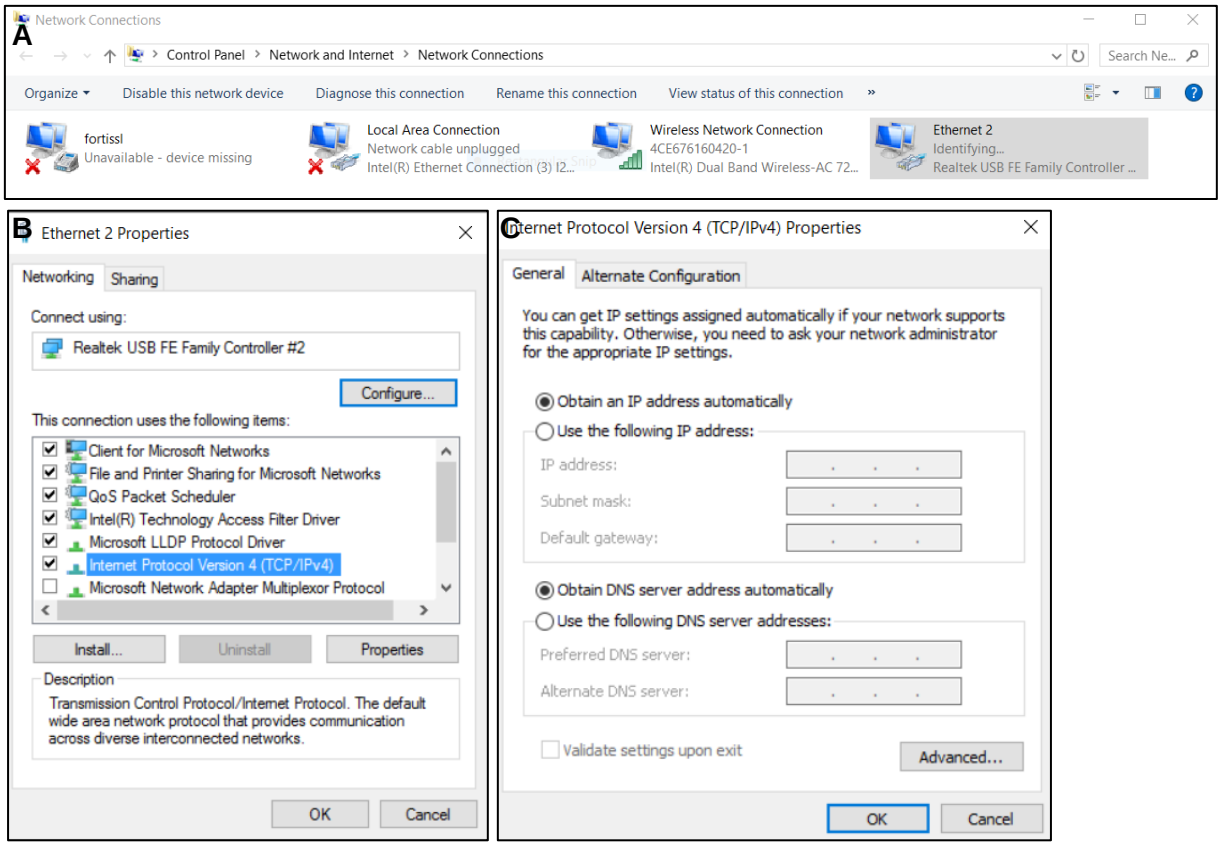
- Connect your instrument to the laptop using the ethernet cable
- Switch the instrument on
- Open the *Network Connections* panel of your PC (*Settings* > *Network & Internet* > *Change Adapter Options*)
- Right-click on the Ethernet port that was previously used to connect to the instrument and select *Properties*. Note: To find the correct port, you can unplug and plug the ethernet cable to see which connection is activated upon switching on the instrument (Fig. A)
- From the list, select *Internet Protocol Version 4 (TCP/IPv4)* > *Properties* (Fig. B)
- Change the dialog exactly as shown in Figure C (Obtain IP automatically).
- Confirm with *OK,* close the dialog with *Close*
- Connect the Ethernet-to-USB adapter to your PC using a free USB port.
- Finish the procedure by configuring the Ethernet-to-USB connection using section *4.5.2 Initial setup of USB port for Ethernet-to-USB connection.*



4.5.4 Change from Ethernet-to-USB to Ethernet

Follow this procedure if you used the Ethernet-to-USB connection and want to change to Ethernet-only.

- Connect your instrument to the laptop using the ethernet cable and Ethernet-to-USB adapter
- Switch the instrument on
- Open the *Network Connections* panel of your PC (*Settings* > *Network & Internet* > *Change Adapter Options*)
- Right-click on the Ethernet port that was previously used to connect to the instrument and select *Properties*. Note: To find the correct port, you can unplug and plug the Ethernet-to-USB adapter to see which connection is activated upon switching on the instrument (Fig. A). The correct port has the description "*Realtek USB FE Family Controller*"
- From the list, select *Internet Protocol Version 4 (TCP/IPv4)* > *Properties* (Fig. B).
- Change the dialog exactly as shown in Figure C (Obtain IP automatically).
- Confirm with *OK,* close the dialog with *Close*
- Connect the Ethernet cable to your PC
- Finish the procedure with configuring the Ethernet connection using section *4.5.1 Initial setup of Ethernet port for Ethernet connection.*



4.6 AmphaChips

The AmphaChips are the core of the impedance measurements. They contain precisely fabricated microfluidic and microelectronic structures and should be handled with care.

4.6.1 Chip Types

Amphasys offers a range of different chip types for a variety of applications. The currently available chip types and the corresponding fluidic specifications are listed in the following table. More information about sample preparation is available in chapter *5 Measurement*.

Chip type	Chip channel dimensions (µm x µm)	Fluidic set	Measurement pump speed (rpm)	Average flow (µl/min) with H_2O
A	15 x 15	S	10	3
B	30 x 30	M	35	18
C	50 x 50	M	80	40
F	80 x 80	L	35	200
D	120 x 120	L	80	460
E	250 x 250	L	350	2400
G	400 x 300	L	650	3500

4.6.2 Chip Handling and Storage

Please store AmphaChips in a dark, dry and dust-free environment at room temperature. Do only touch the black plastic frame and do not touch the glass part containing the microelectrodes. More information about chip cleaning and storage can be found in section *6.3 Chip* .

4.6.3 Buffer Compatibility with AmphaChips

AmphaChips are compatible with the following liquids, which are delivered filtered or should be filtered with maximal 2 µm filter (0.2 µm pore size recommended):

- AmphaFluids, AmphaCalib
- AmphaClean
- PBS or diluted PBS
- Deionized H_2O

- Addition of up to 0.005 % NaN_3
- Addition of up to 0.05 % Tween 20 or Tween 80.

Please make sure that you clean and rinse the instrument with the chip before shutting down and removing the chip. This ensures that the chip does not contain any other fluid than deionized water or air. Another method of cleaning the chip is using deionized water and the Chip Wash Station (section *6.3.2 Chip Cleaning*).

Please be aware that you are working at your own risk when using other buffers than recommended. Examples for known or potentially damaging conditions are:

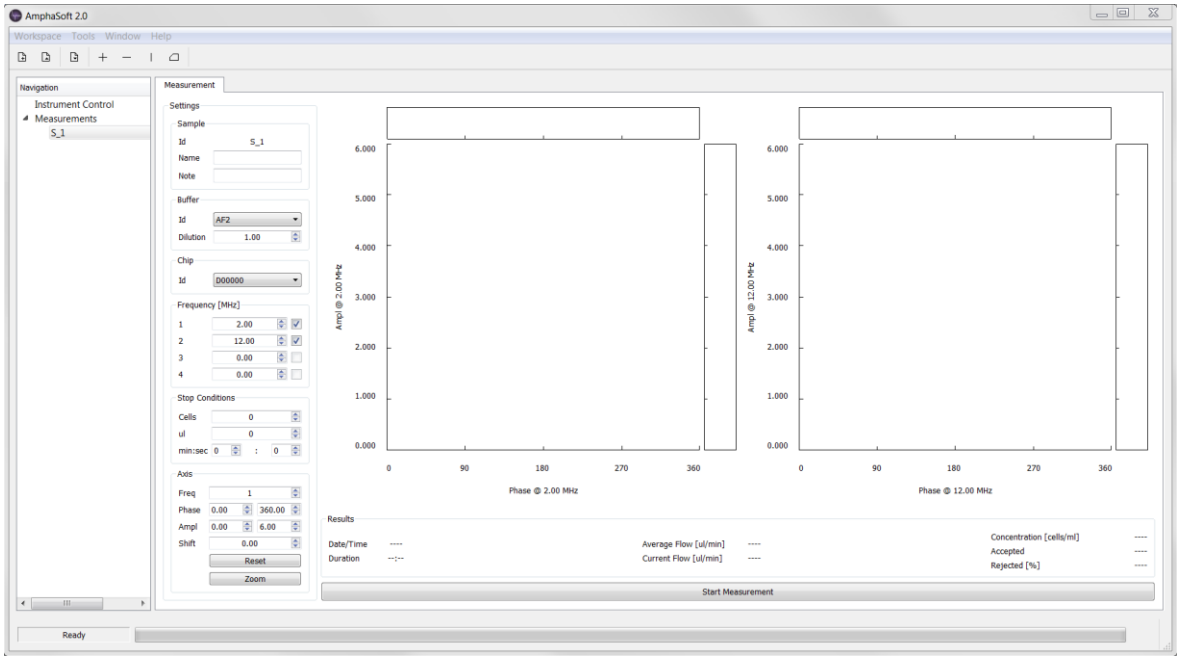
- High (> 8) and low pH solutions (< 6)
- Solutions with high salt or detergent concentrations
- Alcohols, organic solvents or any kind of corrosive liquids

5 Measurement

This section provides information about instrument and software start and sample preparation. Please refer to the *AmphaSoft 2.0 User Guide* for detailed instructions about measurement settings and Software updates. In addition, Amphasys provides *Quick Guides* for download on the website.

5.1 Instrument and Software Start

- Check liquid bottles: The front bottle should contain deionized water for rinsing and cleaning purposes. Please make sure that the liquid levels in the water bottle are always sufficiently high and that the water filter is always sufficiently submerged. The bottle in the left compartment is the waste container and should be empty.
- Check that the instrument Ethernet port is connected to your PC via Ethernet or Ethernet with Ethernet-to-USB adapter.
- Start up your computer if not started yet.
- Switch on the instrument. The power switch is located at the rear panel of the instrument.
- Start the software AmphaSoft 2.0 by double clicking the icon on your desktop.



- You will face the AmphaSoft 2.0 main GUI.
- A connection will be established automatically, when the first operation is performed. Alternatively, click on *Instrument Control* in the *Navigation* panel and then select the *Admin* tab. In the connection section, click *Connect*. The software will now connect to the instrument.

5.2 Experiment Setup

Perform an initial rinsing step to clean the instrument fluidic system and set up your experiments by choosing measurement settings and adding measurements to your workspace. You will find detailed information about these tasks in the *AmphaSoft 2.0 User Guide* and the Amphasys *Quick Guides* on the website.

5.3 Sample Preparation

After rinsing the system and setting up your measurements, prepare your samples. Use the recommendations below as a starting point and have a look at our *Sample Preparation Quick Guide* on the website.

5.3.1 Creation of a Single Cell Suspension

The Ampha Z32 Impedance Flow Cytometer can measure electrical properties of single cells in suspension. Therefore, a single-cell suspension in a suitable buffer is required. The buffer choice is crucial. For pollen measurements, Amphasys provides an extensive list with information about pollen and corresponding measurement buffers (see Pollen Analysis Instructions on our website). For other applications, consult our application specialists. (support@amphasys.com).

- A typical measurement protocol involves resuspending the cell material in an appropriate buffer in a microcentrifuge tube. Depending on your application gently shake, pipet or vortex the tube to disperse cells.

5.3.2 Filtration and Dilution

In order to prevent clogging of the chip, filter your single cell suspension prior to measuring. Amphasys recommends the use of filters that have pore sizes of maximally half the characteristic channel dimension (e.g. max. 60 µm filters for 120 µm D-chip).

- In a typical sample preparation protocol, a filter is placed on top of 5 ml round-bottom tubes, and the content of the single-cell suspension is poured through the filter.
- Additional buffer can be added to the tube in order to dilute it.

Notes

1. Please make sure that you do not introduce a bias to your measurements by choosing too small filters. It is good practice to check your sample under the microscope to determine the size of the largest particles of interest.
2. Chip blockage is mainly caused by two factors. First, large particles (e.g. particle clumps or aggregates) have a higher chance of clogging the measurement channel inlet. This factor can be eliminated by filtering appropriately. Second, clogging occurs in a concentration-dependent manner. Highly concentrated samples have a higher chance of clogging. If you have chip clogging issues, reconsider your chip and filter choice and dilute your samples.

The following table provides recommendations of cell concentrations and filter pore sizes for all AmphaChips.

Recommended cell concentrations and filter pore sizes for each chip type

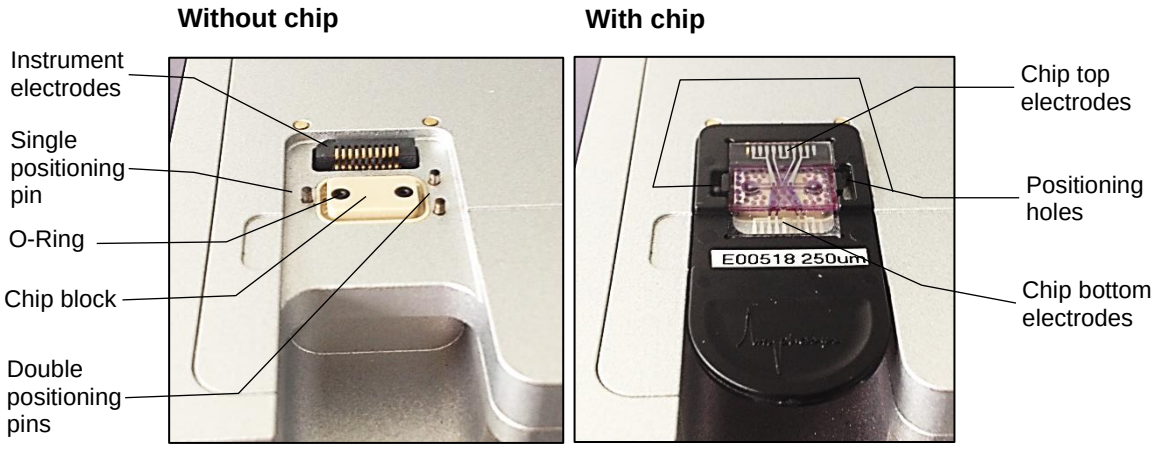
Chip type	Chip channel dimensions (µm x µm)	Maximum cell concentration (max. 1000 cells / second as a rule of thumb)	Filter pore size
A	15 x 15	30 Mio per ml	5 or 10 µm
B	30 x 30	5 Mio per ml	20 µm
C	50 x 50	3 Mio per ml	30 µm
F	80 x 80	300’000 per ml	50 µm
D	120 x 120	130’000 per ml	50 or 100 µm depending on pollen size
E	250 x 250	25’000 per ml	100 or 150 µm depending on pollen size
G	400 x 300	3’000 per ml	200 or 300 µm depending on pollen size

5.4 Chip Placement

The Ampha Z32 Impedance Flow Cytometer and the AmphaChips have two fluidic and two electronic interfaces. For a successful measurement, all four interfaces must be connected properly. Therefore, a correct chip placement is crucial. For that purpose, the AmphaChips are designed with positioning holes that help to align the chip electrodes with the instrument electronic pins. In addition, the chip holes are aligned with the tubing system. In addition, the chip is pressed on the chip block by a spring integrated in the instrument lid.

In order to place a chip, open the instrument lid. Place the chip using the 3 positioning pins (Figure). The single pin fits into the small positioning hole on the left hand side of the chip, and the double positioning pins fit into the larger positioning hole on the right hand side. This mechanism ensures that the chip only fits in one direction (facing up).

Note AmphaChips are precisely fabricated microfluidic and microelectronic devices. Please do not apply force when placing the chip and only touch the plastic frame of the chip with your fingers. Do not close the lid when the chip is not positioned correctly.



5.5 Measurement

After sample preparation and chip placement, the samples are ready for measurement. Attach the samples to the sample holder and start the measurement from the AmphaSoft 2.0 interface by clicking *Start Measurement* in the *Measurement* tab. Please also have a look at our *Measurement Quick Guide* on our website.

Consider the notes below when measuring quickly sedimenting cells (e.g. for E and G chip), or when preparing large series of measurements.

Note

***Sedimentation*:** As many cell types have a higher density than the measurement buffers, they will continuously sediment to the bottom of the vial. This will lead to inaccurate concentration measurements. Please make sure that you mix samples well directly before measuring. In addition, samples can be mixed during measurement by tapping at the sample containers. Using Amphafluids AF8 or AF9 prevents or delays the sedimentation.

For accurate cell counting have a look at the Counting mode described in the *Counting Quick Guide* and the *AmphaSoft 2.0 User Guide*.

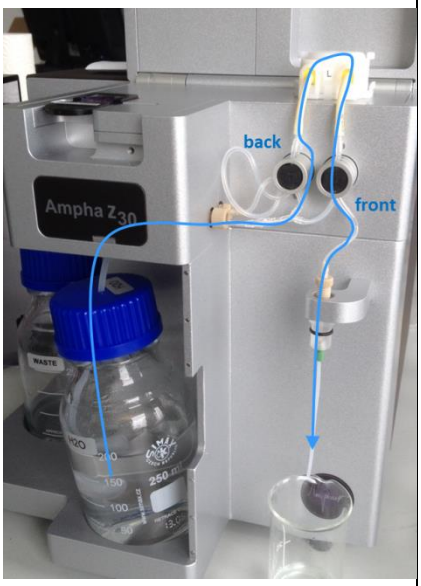
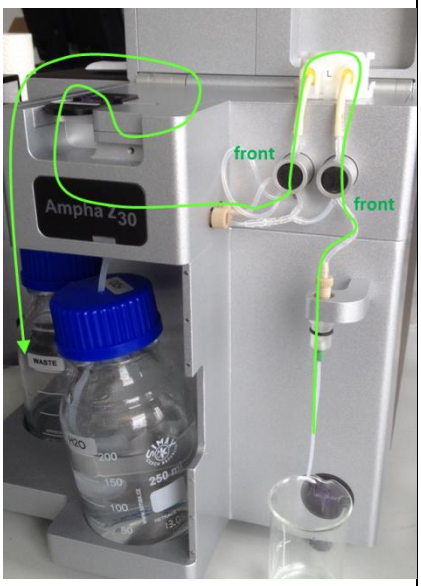
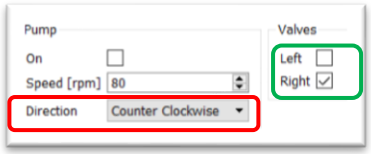
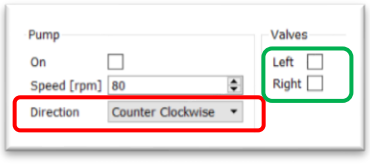
***Measurement Series*:** Amphasys recommends measuring samples immediately after preparation. If you intend to prepare a large series of samples, please check the buffer compatibility with your sample (e.g. influence of suspension time on cell viability). Make sure that you don't bias your measurement series by incubating sensitive samples for various times in the measurement buffer.

6 Maintenance

This chapter covers Ampha Z32 maintenance. Please follow the instructions carefully in order to ensure optimal instrument performance and longevity. The maintenance steps are also described in the *Cleaning and Maintenance Quick Guide* on our website.

6.1 Ampha Z32 Fluidics

The Ampha Z32 fluidic system consists of tubings and drillings made from different materials, which are getting in contact with the biological samples that are measured. Many biomolecules and microorganisms can attach to surfaces and form adsorbed layers or biofilms. Therefore, it is inevitable to keep all fluidic lines clean. There are several fluidic maintenance protocols that should be followed. The initiation of these protocols is described in the *AmphaSoft 2.0 User Guide*.

A. Rinsing/Flush Part 1	B. Rinsing/Flush Part 2	C. Measurement (loading)
		
		
Pump – clockwise Valve - left active	Pump – counter clockwise Valve - right active	Pump – counter clockwise Valve - none active

6.1.1 Initial Rinsing

Prior to each measurement series, a rinsing cycle should be performed. This quick and automated protocol cleans all fluidic paths with deionized water from the water bottle. Please make sure that you place a liquid container (e.g. beaker or 5 ml round-bottom tube) under the sample holder to collect rinsing water. For the initial rinsing cycle, you can use either a measurement chip or a cleaning chip. Select this in the AmphaSoft *Measurement* tab. You can start the cleaning protocol from the AmphaSoft 2.0 *Basic* tab.

6.1.2 Instrument Cleaning

The instrument cleaning cycle should be performed as the last part of the daily measurement routine. The cleaning cycle is a combination of rinsing steps with deionized water and AmphaClean solution. AmphaClean solution is a special cleaning liquid containing detergents. Please make sure that you place a liquid container (e.g. beaker or 5 ml round-bottom tube) under the sample holder to collect rinsing water. For the cleaning cycle, you can use either a measurement chip or a cleaning chip; select this in the AmphaSoft *Measurement* tab. Start the cleaning protocol from the AmphaSoft 2.0 *Basic* tab. Please make sure that measurement chips are not exposed for prolonged periods (e.g. overnight) to AmphaClean.

6.1.3 Instrument Disinfection

The instrument disinfection protocol should be performed at least once per week or when working with contaminated material, fungi or bacteria. Please exchange the deionized water before starting the disinfection protocol. This protocol requires 70 % ethanol, preferably in a 5 ml round-bottom tube.

Attention! Never perform the instrument disinfection protocol with measurement chips. **Only** use the **Cleaning Chip** for instrument disinfection and select it in the AmphaSoft *Measurement* tab.

Cleaning Chip

6.1.4 Water and Waste Bottles

Exchange the deionized water in the water bottle on a regular basis. Amphasys recommends the use of fresh deionized water every day. To prevent the growth of microorganisms, you can add sodium azide (NaN_3) to the water bottle at final concentrations of 0.001% to 0.005% **(very toxic compound! Use health and personal protection tools).**

Note When the water filter is dry or contains air bubbles, it may take a while until water is aspirated through the tubing. Perform a few rinsing steps until the tubing is filled with deionized water.

Please empty and clean the waste bottle daily to prevent growth of microorganisms. If the waste tubing is in contact with contaminated waste liquid, microorganisms can invade the tubing set and cause damage to the instrument. It can be beneficial to shorten the waste tubing to prevent it from contacting the waste liquid.

6.2 Quarterly and Yearly Maintenance

Amphasys recommends a quarterly maintenance of the external fluidics of the instrument at normal use and shorter cycles at heavy use. This maintenance includes the exchange of the silicone tubing, the peristaltic pump head and O-rings. For the yearly maintenance we recommend a complete instrument service from an Amphasys assistant or official distribution partner. The yearly service consists of an operational check of the instrument and the maintenance including the exchange of the complete internal fluidics and the water filter.

Omitting the maintenance guidelines may cause limited warranty.

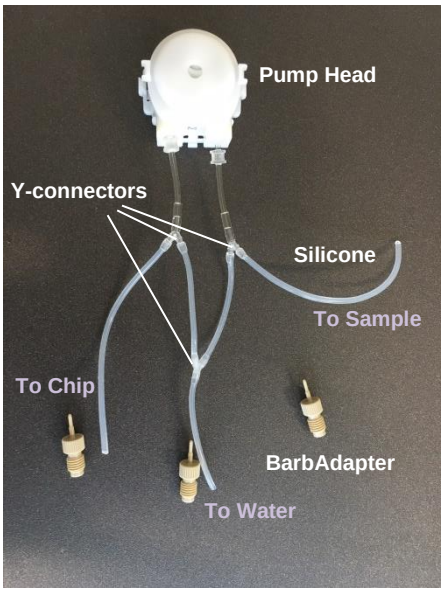
For any details please contact us (https://amphasys.com/ampha-z32-pollen-analyzer/#support).

The following table lists the parts and reagents that are required for regular maintenance. More details are available at https://amphasys.com/ampha-z32-pollen-analyzer/#consumables .

Part Number	Description
12.100	Tubing Set L for 80 µm, 120 µm, 250 µm and 400 µm chips
12.030	Tubing Set M for 30 µm and 50 µm chips
12.015	Tubing Set S for 15 µm chips
19.012-S	Sample Aspiration Tubing S
19.012-L	Sample Aspiration Tubing L
21.000	AmphaCalib
21.900	AmphaClean (Cleaning buffer)
11.900	Cleaning Chip
11.901	Chip Wash Station

6.2.1 Tubing Set Replacement

The Ampha Z32 Impedance Flow Cytometer is equipped with one of three different tubing sets (L, M and S). The tubing set consists of a peristaltic pump head, silicone tubings, Y-connectors and barb adapters (Figure). A tubing set replacement involves the exchange of the pump head and the silicone tubing (including Y-connectors).



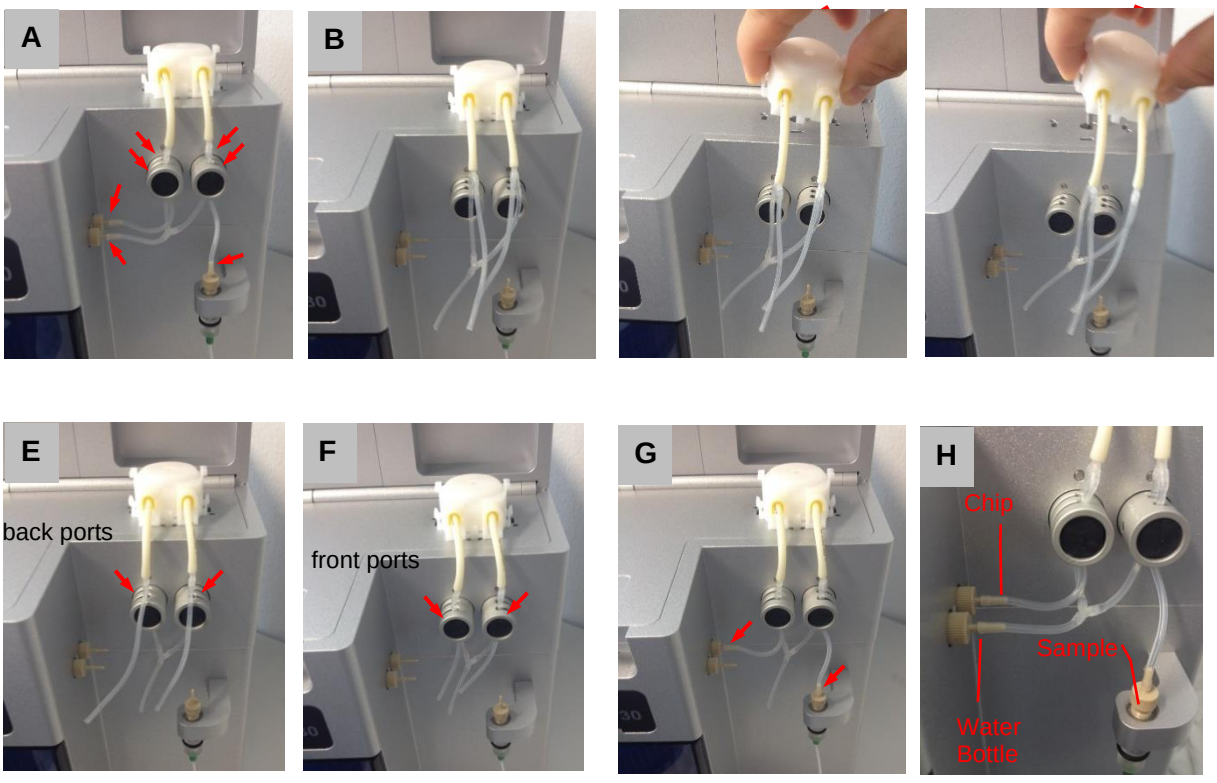
Replacement Procedure (images below)

- Make sure that the pump is not running. The status of the pump is indicated in the AmphaSoft 2.0 *Advanced* tab in the section *Pump*.
- Remove the three silicone tubing ends from the barb adapters (Fig. A).

- Pull the tubing out of the two pinch valves. As each of the pinch valves compresses one of the two inserted tubing's, the removal of one of the tubing requires force while the other one can be removed easily (Fig. A, B).
- Remove the pump head by pressing on the two lateral clamps using thumb and forefinger (Fig. C).
- Place the new pump head on the pump motor pin and press it down until the clamps engage (Fig. D).
- Push/Pull the two tubing segments that will connect to the water bottle into the back ports of the pinch valves. Make sure they are properly positioned (Fig. E).
- Push/Pull the two tubing segments that will connect to the chip and to the sample into the front ports of the pinch valves. Make sure they are properly positioned (Fig. F).
- Connect the three silicone tubing ends to the barb adapters (Fig. G, H).
- Start the instrument and perform 2-3 rinsing cycles to fill the fluidic system with water.

Note The rinsing cycles can be used to verify that the tubing replacement was successful. First, inspect the tubing connections for leakage (e.g. Y-connectors, barb adapters etc.). Ensure the proper positioning of the silicone tubing by checking the liquid flow direction during a rinsing cycle: The rinsing cycle consists of a flushing of the sample aspiration tubing and a flushing of the fluidic lines to the waste. Therefore, water should first come out of the sample aspiration tubing and then (after valve switching), out of the waste tubing. These two processes are repeated once.

Tubing Set Replacement



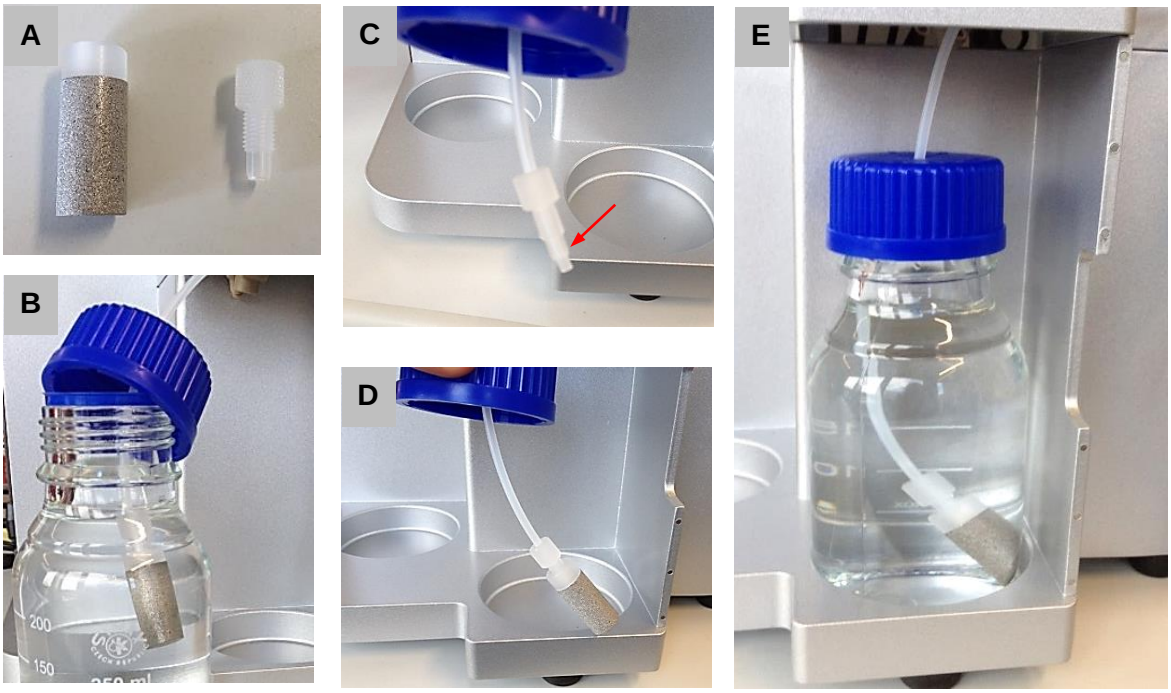
6.2.2 Water Bottle Filter Replacement

The water aspiration tube is equipped with a filter (Fig. A). This filter should be replaced on a yearly basis. Please use gloves to handle water filters.

- Open the water bottle, remove it and put it aside (Fig. B)
- Unscrew the filter from the fitting (Fig. C). Check that the tubing in the fitting has overhang of about 4 mm.
- Mount the new filter onto the fitting. Make sure it fits tightly (Fig. D)
- Fill the water bottle with fresh deionized water.
- Place the tubing with the new filter back into the water bottle (Fig. E).
- Perform rinsing cycles until the Ampha Z32 fluidic lines are filled with deionized water.

Note When the water filter is dry or contains air bubbles, it may take a while until water is aspirated through the tubing. Perform a few rinsing steps until the tubing is filled with deionized water.

Water Bottle Filter Replacement



6.3 Chip Maintenance

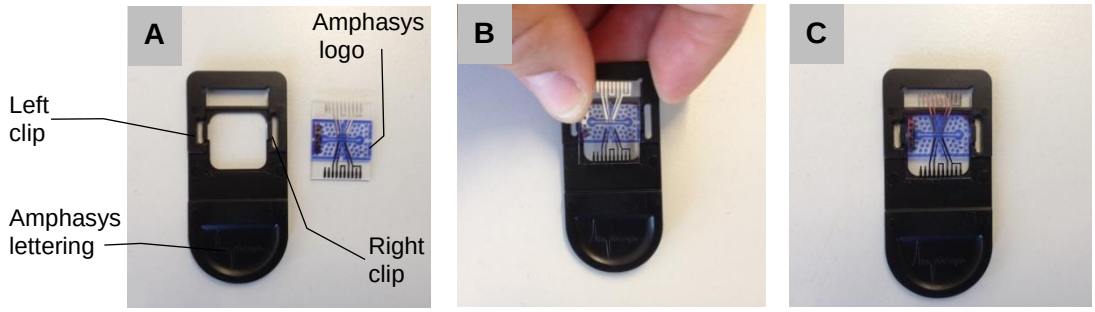
The AmphaChips are the core of the impedance measurement. They are delicate microfluidic devices. They should be handled with care, cleaned regularly und stored in dry and dust free box in the dark and at room temperature. The following sections cover chip assembly, cleaning, storage and deblocking.

6.3.1 Chip Assembly

AmphaChips consist of microfluidic and microelectronic structures that are supported by a glass sandwich. This glass part is embedded in a plastic frame. In case the glass core jumps out of the frame, just put it back in place using the following instructions.

Chip Assembly

- Place the plastic chip frame in front of you, with the Amphasys lettering facing towards you (Fig. A).
- Place the chip with inlet and outlet holes facing down next to the chip frame. The Amphasys logo should be in the central right part of the glass.
- Keeping that orientation put the chip into the holder by carefully pressing it first into the left lateral clip (Fig. B) and then into the right lateral clip (Fig. C) until the chip is snapping in its place.



6.3.2 Chip Cleaning

- The chip surface can be cleaned with a lint-free tissue (e.g. Anticon® wipes) and water, if needed.
- Chips should be cleaned immediately after use. There are *Flush*, *Rinse* and *Cleaning cycles* that can be used while the chip is inserted in the instrument (see *AmphaSoft 2.0 User Guide*). Alternatively, the chips can be cleaned outside of the instrument in the chip wash station with filtered high-purity water or mild detergents (see below).

  When using the chip wash station, the chip cleaning should be done from the inlet hole to the outlet hole (for chips that are not clogged). Use the instructions in section *6.3.4 Using the Wash Station to Unclog Chips* for unclogging clogged chips.

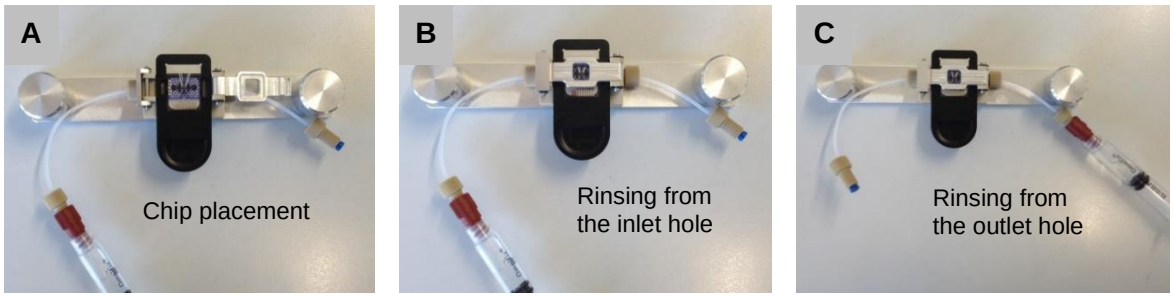
Attention! Do not apply high pressure and do not use liquids with pH < 6 and pH > 8, organic solvents or any corrosive agents. This can lead to the destruction of the chips.

6.3.3 Chip Storage

Chips should be stored in a clean state and in a dark and dust-free environment.

6.3.4 Using the Wash Station to Unclog Chips

When chips are clogged by dust, cells or other particles, we recommend the cleaning procedure with the chip wash station (Fig. A). In most cases, applying soft pressure from the outlet hole (Fig. C) will remove the clogging immediately.



Cleaning should be started with < 0.5 µm filtered high-purity water. If necessary, other detergents can be used (table). Don't rinse with any organic liquids like alcohols.

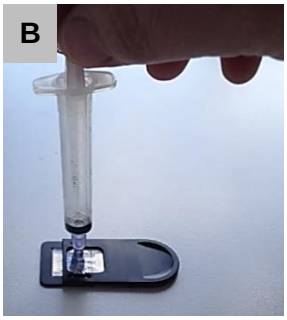
- Checking the chip under a microscope usually helps to identify the cause of the clogging. In case of a clogging between inlet and the sensing channel, rinse from the outlet hole (Fig. C). If the clogging is between outlet hole and sensing channel, rinse from the inlet hole (Fig. B).
- After special cleaning procedures always rinse the chip with water from the inlet hole (Fig. B) and remove the water afterwards by pushing air through the chip wash station using a syringe filled with air.
- Detergents can be warmed to about 40 degrees for better cleaning effects.

Detergent	Usage
<0.5 µm filtered high-purity **water**	General cleaning liquid
<0.5 µm filtered 0.5% **Neutracon** or solutions with low amount of dishwashing liquids (room temperature or slightly warmed)	Cleaning of persistent biological cell blockings or residues in channels and holes. Detergents should be used for a maximum of 10 minutes, because electrodes could erode after extended exposure.
<0.5 µm filtered **AmphaClean**	Cleaning of dirt and persistent biological cell blockings or residues in channels and holes for a maximum of 10 minutes.

Additional tips for resistant cloggings

- After cleaning with water, let the chip dry overnight and try to clean again.
- Place the chip for 1 minute in an ultrasonic bath (containing clean water), then put it into the wash station and try to clean again.
- Cleaning of the chip holes and channel with water or air can be achieved using a normal plastic syringe with a small silicon tubing fitted on top of the syringe tip (Fig. A). Press the

silicone tubing onto either chip inlet or outlet and apply positive or negative pressure by pressing or pulling the plunger (Fig. B).



- If you know what kind of dirt is blocking the channel, you may try to dissolve the dirt with a material-specific solution or detergent. Keep in mind that the chip, channel structure and electrodes can be destroyed by certain chemicals. If you are in doubt about the compatibility, please contact Amphasys.

6.4 Valves

6.4.1 Valve Checking

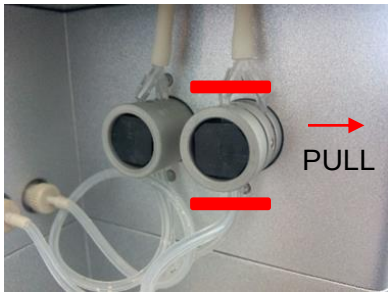
The valves are in the front part of the Ampha Z32 and are an important part of the fluidic system of the machine. We recommend testing the valve performance before starting measurements by clicking the valve checkboxes in the *Advanced* tab. A loud “click” sound indicates proper functioning of a valve

6.4.2 Valve Cleaning

Due to leakage, the valves can be blocked by crystallized sugars and salts contained in the buffers. That can cause that no liquid arrives at the chip, and therefore no cells are detected by the system. Due to an accumulation of residues, the valves might not switch and therefore the fluidic system of the Ampha Z32 may not work properly. Moreover, a non-optimum performance of the fluidic system could cause air bubble to appear inside the fluidic lines.

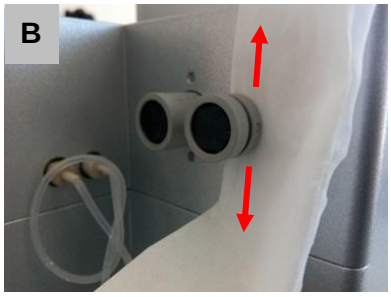
Cleaning

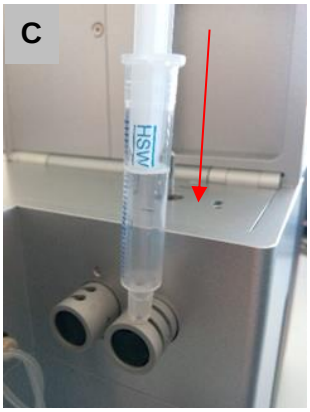
- Switch off the machine and place it somewhere away from any electrical device or power supply point.
- Hold the silicone tubing with the two hands from the points that are marked in the picture below and pull the silicone tubing out of the valve’s grooves.

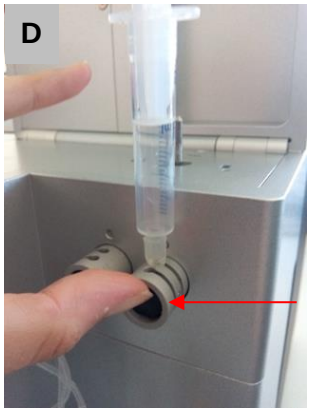


- Repeat it for the second valve.

- Take a wipe and soak it with deionized water. If the deionized water is a bit warm (35-45 °C), it would dissolve better the residues (Fig. A).
- Introduce the wet wipe several times inside the grooves of the valves and move it inside the valve's groove up and down to unblock the groove and dissolve the salts and sugars (Fig. B).





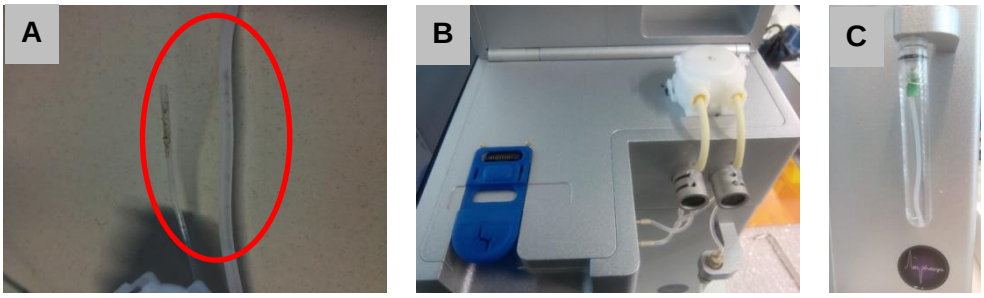


- Take the plastic syringe with a small silicone tip (in the starter cleaning pack) filled with deionized water and flush water through the valve grooves (Fig. C). Absorb the water flowing through with a tissue at the bottom side.
- Push the valves smoothly in and out with a finger while flushing water through (Fig. D).
- Finally, take a small pipette tip and remove the sugar and salts residues that remain in the front part of the valve. Carefully move the pipette tip around the frame of the valve (Fig. E).
- Take a dry wipe and introduce it in all the grooves of the two valves to dry them.

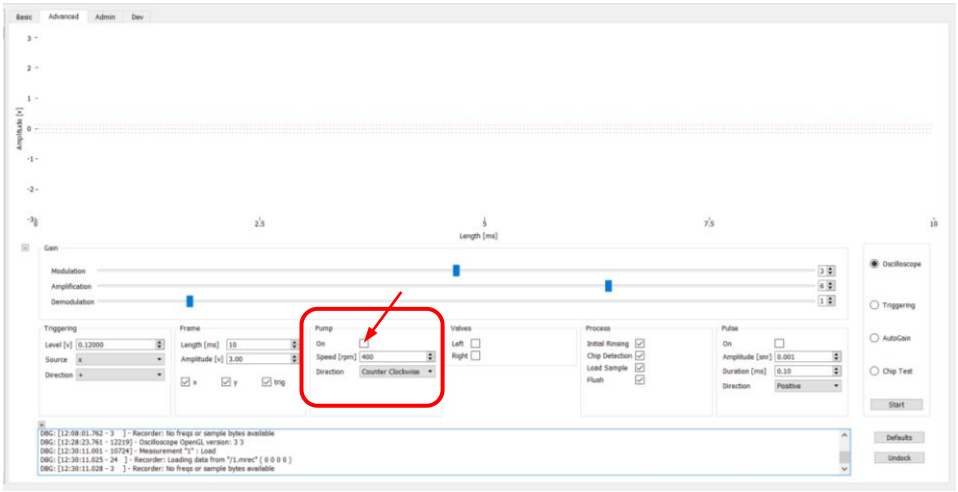
6.5 Fluidic Set Cleaning

Due to insufficient instrument cleaning and deposition of sugars in the tubings, fungus can grow inside the fluidic system of the Ampha Z32. A good cleaning routine after every working day prevents this problem. To prevent the growth of microorganisms, you can run the disinfection protocol using the Cleaning Chip several times. Consult chapter 6.1 for fluidics and cleaning protocols. In case of fungus growth in the tubing, please follow the procedure described below.

- A contaminated tubing infected with fungi is visible in Fig. A.
- Take warm deionized water and 70% ethanol; fill 4 ml of each in a 5 ml Falcon tube.
- Insert the cleaning chip into the instrument (Fig. B).
- Attach the water sample at the sample holder (Fig. C).



- Set the pump speed in the AmphaSoft/Instrument Control/*Advanced tab* at 400 rpm



- Switch on the pump and let all the deionized water go through the fluidic system, and then switch off the pump.
- Attach the 70 % ethanol sample to the sample holder and set the pump speed to 150 rpm.
- Switch the pump on and let all the 70% ethanol go through the entire fluidic system
- Repeat the ethanol rinsing step once.
- Put another 4 ml of deionized water (at 45 °C) in a Falcon tube and set the pump speed to 400 rpm. Switch on the pump and let all the deionized water go through the fluidic system, switch the pump off.
- Perform two normal rinsing cycles and the system is ready for measurement.

Attention! In case of severe growth of fungi inside the fluidic lines, ethanol should be left overnight in the lines. In case of questions due to extreme fungi growth, please contact Amphasys or your local distributor.

6.6 Flow Sensor

If maintenance procedures are not strictly followed, the flow sensor can be contaminated due to salt and sugar deposition or fungal growth. In this case, a reduction of the average liquid flow rate can be observed. Reference values of the average flow rate are provided in the table in section 4.6.1.

6.7 Peristaltic Pump

The peristaltic pump head should be exchanged every 3 months, if the instrument is used regularly. Otherwise, the correct performance of the pump head is not guaranteed.

The motor of the peristaltic pump should not show any problems during normal usage of the machine. If you think that the motor is not turning properly, please contact Amphasys or your local distributor.

7 Autosampler

7.1 Specifications

The Amphasys autosampler is an instrument that allows automated measurements of up to 192 samples in a single run. Supported are 96-well deep well plates (2 ml) in combination with AmphaChips F, D, E and G (80 µm, 120 µm, 250 µm and 400 µm). The following table provides an overview of the experimental setup and restrictions.

Item / Parameter	Specification
Chip Types	F (80 µm), D (120 µm), E (250 µm) and G (400 µm)
Microtiter Plate Formats	• Measurement: Deep well 96-well, 2 ml (2.2 ml) capacity, maximum plate height = 44 mm. U-type preferred. • Cleaning, Disinfection and Chip Test: 12-well plate
Sample Volume	Max. 1.8 ml liquid per well of the 96-well plate
Buffers	All Amphasys measurement buffers. Adding detergents (e.g. Tween 20) or other additives to the buffers is not recommended, as this leads to foaming
Stop Conditions	At least a µl (microliter) stop condition must be set. Please avoid aspiration of air after the sample finishes. Also consider the dead volume at the bottom of the well that the needle cannot reach
Number of Samples	1 – 192 samples per run. Note that after 96 samples you may need to empty both waste bottles and refill both water bottles.

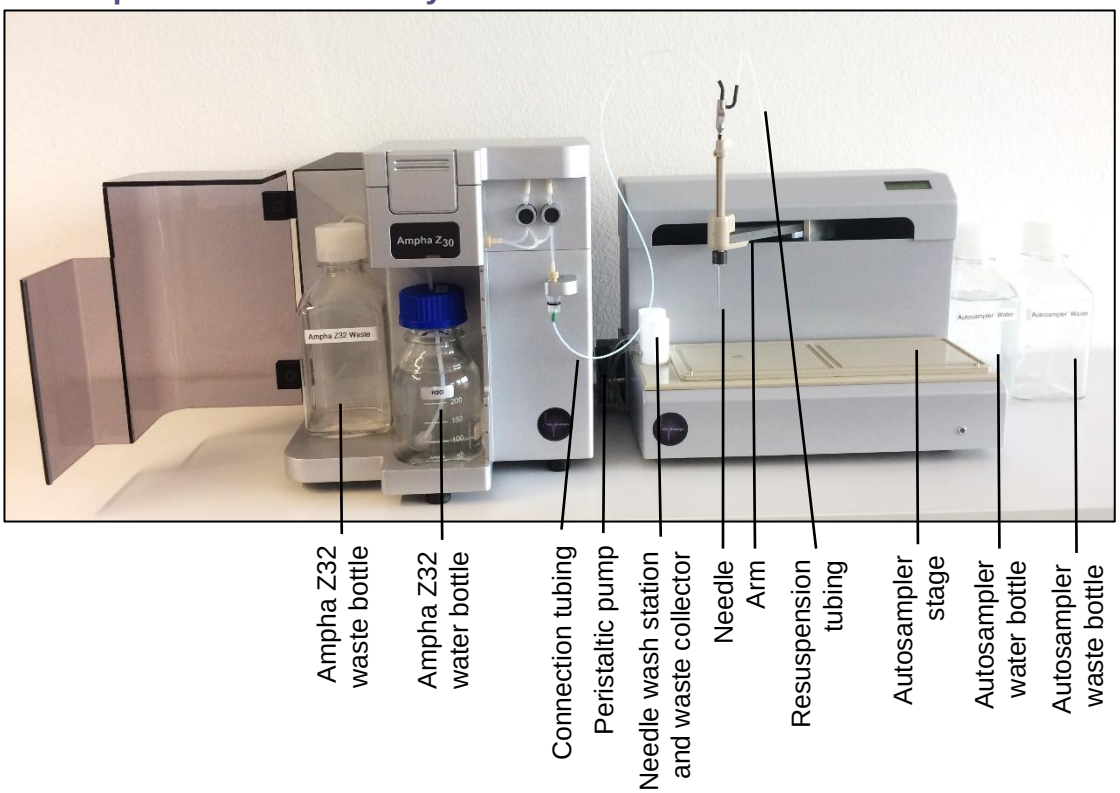
7.2 Description and Features

7.2.1 Parts

The autosampler consists of a stage supporting two 96-well microtiter plates. Each well of these plates can be addressed by a needle. This needle has a double functionality. First, it is used to aspirate the sample and second, it releases air to the bottom of a well to resuspend sedimented particles. The air flow is generated by a peristaltic pump. To prevent carryover, a needle wash station is available to automatically clean the needle after each measurement. A water bottle provides fresh

water to the needle wash station and a waste bottle collects waste from the waste collector. Liquids are transported to and from the waste and water bottles by the same peristaltic pump that is used for sample resuspension. Prior to each measurement, an air gap is introduced to the needle tip to further reduce sample carryover. A clogging detection algorithm is constantly monitoring fluidic processes and can stop a measurement series in case of a chip clogging.

7.2.2 Resuspension Functionality



The autosampler features a sample resuspension functionality. After submerging the aspiration needle in the sample solution, air bubbles are released from the needle tip while the needle performs a linear bidirectional movement. This leads to the resuspension of sedimented pollen in the well. After resuspending, the sample is aspirated and loaded into the chip for measurement.

7.2.3 Clogging and Air Bubble Detection

Clogging of the chip can occur for several reasons:

- Samples not filtered
- Particle concentration too high
- Particles aggregate in solution
- Insufficient cleaning of the fluidic system

To prevent damage to the Ampha Z32 cytometer due to cloggings, two safety features are in place. First, the silicone tubing connecting to the Ampha Z32 internal fluidics can jump from the adapter in

case the pressure exceeds a certain threshold. Second, a clogging detection algorithm constantly monitors the flow rate and stops any process in case the flow drops to zero. Zero flow occurs in case of a chip clogging or if large air bubbles are in the tubing system. Both cases are unwanted and should be resolved immediately. In case the fluidic lines are not yet filled with liquid (e.g. after transportation or maintenance), please ignore clogging detection messages and perform Ampha Z32 instrument rinsings until the fluidics are filled.

7.3 Initial Setup

Please follow the instructions carefully and sequentially.

After receiving the autosampler, unpack it and place it on a flat surface.

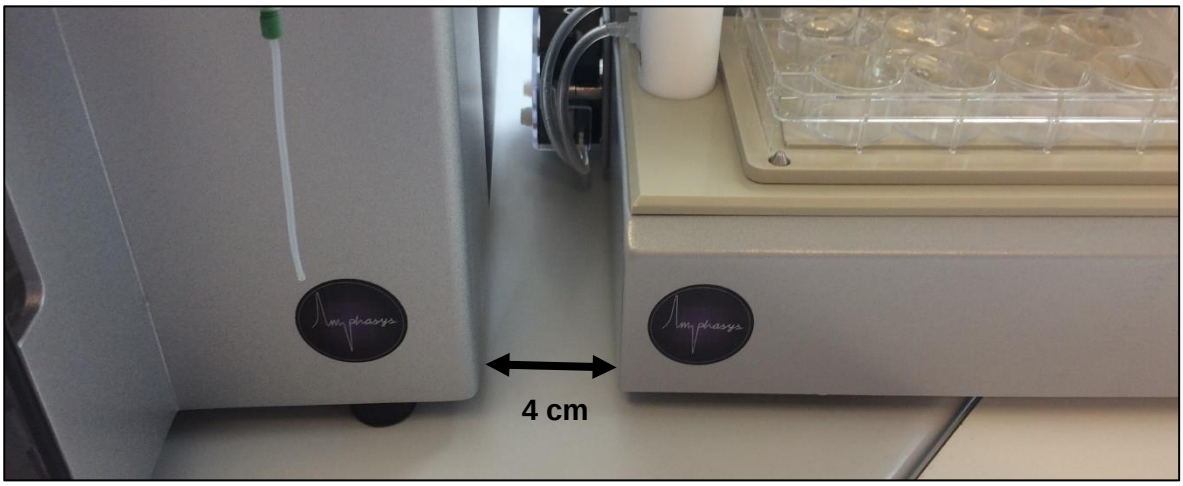
7.3.1 Changing the Laptop Power Settings

Typically, autosampler measurement series can last several hours. Therefore, it is important that the laptop that is running AmphaSoft 2.0 software is not going into sleep mode. Please check the power settings of your laptop and adjust them to prevent sleep mode.

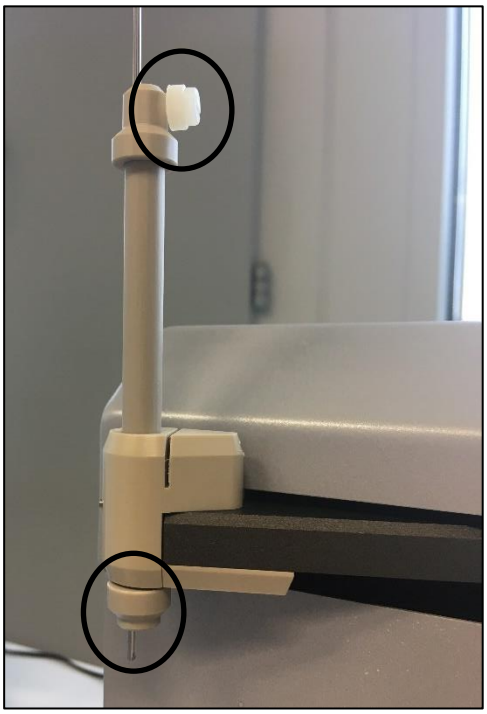
- Open the power settings of your laptop (*settings* > *system* > *power & sleep*)
- In the *Screen* and *Sleep* sections, adjust all settings to *Never* or a very high value that would not impede your measurement series.
- Open the *Control Panel* > *Network and Internet* > *Network and Sharing Center*. Click on *Change adapter settings* in the left panel. In the list of devices that appear, *right-click* on the Ethernet-to-USB adapter (*Realtek USB FE Family Controller…*), select *Properties*.
- In the *Properties* window, click *Configure* and select the *Power Management* tab. Make sure that the box which allows to turn off this device is unchecked.

7.3.2 Autosampler Fluidics

- Place the autosampler at the right side of your Ampha Z32 Impedance Flow Cytometer, approximately 4 cm separated from each other.

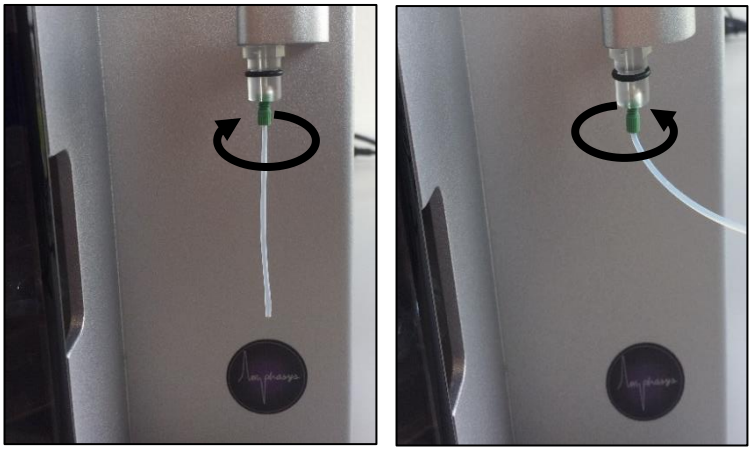


- Insert the needle into the autosampler arm until the needle tip is coming out of the lower cap of the autosampler arm. You may have to open the white screw on the autosampler arm. After positioning, tighten the white screw softly.

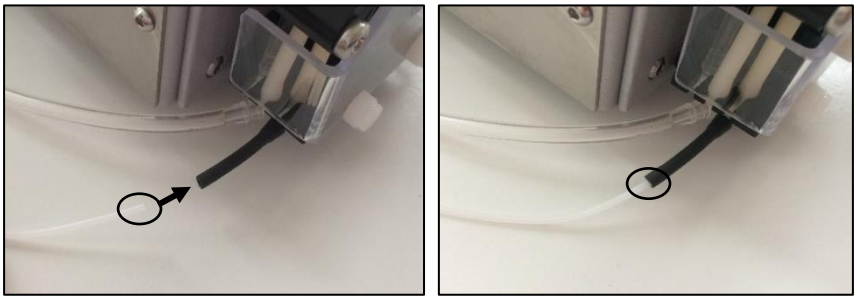


- Then, carefully unscrew the sample aspiration tube of the Ampha Z32 (clockwise if viewed from above)

- Carefully screw the green fitting of the sample tube of the autosampler into the sample tube adapter of the Ampha Z32 (counter clockwise)



- Insert the free end of the resuspension tubing (connected to the needle) into the free black tubing connector on the rear side of the peristaltic pump. Make sure it is connected firmly.

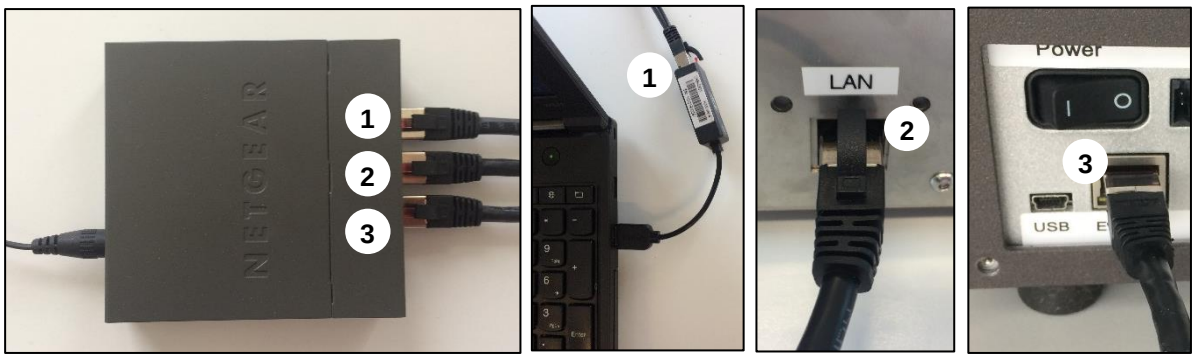


- Place the autosampler water and waste tubing with the attached caps on the corresponding bottles
- Replace the Ampha Z32 waste bottle with the delivered waste bottle

- If necessary, adjust the lid of the peristaltic pump, i.e. adjust the screw to change the pressure that is exerted on the tubing (see also chapter *7.11.5 No Liquid Supplied to Needle Wash Station*).

7.3.3 Communication and Electronics

- Unpack the Ethernet switch and the two Ethernet cables
- Connect the two Ethernet cables delivered with the autosampler and the third Ethernet cable with Ethernet-to-USB adapter (delivered with Ampha Z32) with the Ethernet switch
- Connect the Ethernet cable with Ethernet-to-USB adapter to a USB port of your laptop (1)
- Connect the other two Ethernet cables to the autosampler (LAN port) (2) and to the Ampha Z32 (Ethernet port) (3)

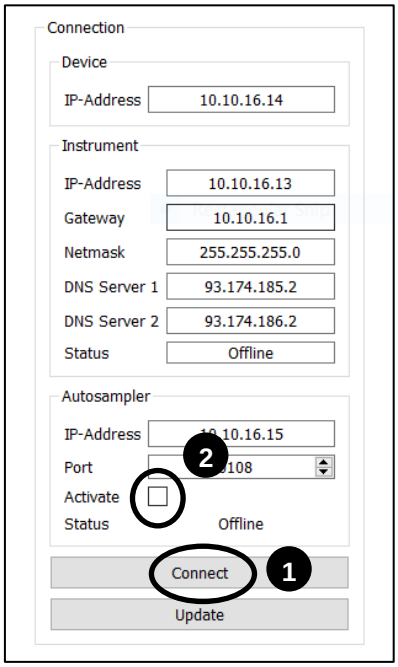


7.4 Starting Up the System

- Make sure all Ethernet cables are connected to their devices (Autosampler, Ampha Z32 and laptop)

- Plug the power adapter of the Ethernet switch into the power supply
- Make sure the autosampler is switched off. Connect the autosampler power adapter to the power supply
- Remove all objects from the autosampler stage
- Switch on the autosampler

Note The autosampler will automatically perform an initialization sequence upon switching on. If there is any object on the autosampler stage which is higher than the specified maximum height, the autosampler needle can crash into the object and this can cause instrument damage.



- Switch on the Ampha Z32
- Open AmphaSoft 2.0
- Open the *Admin* tab and click *Connect* in the *Connection* section (*Image step 1*). This step will establish a connection between your laptop and the Ampha Z32. A successful connection is indicated with the status *Online* in the *Instrument* section.
- After successful connection to the instrument, click the *Activate* check box in the *Autosampler* section (*Image step 2*). A successful connection is indicated with the status *Online* in the *Autosampler* section.

7.5 Adjusting the Needle Position

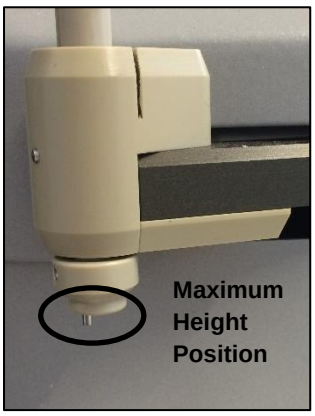
Adjusting the needle position is important, as it prevents needle crashes and reduces dead volume. The needle position must be adjusted for each of the following situations:

- After transportation (including initial setup)
- After exchanging a needle
- After a needle crash
- When a new plate format is used

Please refer to the following instruction to adjust the needle position:

- Make sure that the autosampler is switched on and activated (*Online* status in the *Autosampler* section of the *Admin* tab in AmphaSoft)
- Place a 96-well plate (2 ml deep well) at the left position of the autosampler stage

- In the AmphaSoft menu, select *Tools* > *Autosampler* > *Commands* > *Needle Adjustment.* This will launch the Needle Adjustment Wizard.
- In the first dialogue you will be asked to fix the needle at the maximum height position. Open the white screw of the autosampler arm and pull the needle up until the tip is at the needle opening of the bottom cap of the autosampler arm. Fix the needle at this position using the white screw.
- Follow the instructions of the Needle Adjustment Wizard
- Once the needle is fixed, perform an initialization sequence by selecting *Tools* > *Autosampler* > *Commands* > *Initialize*



7.6 Filling the Autosampler Water Tubing

The autosampler water tubing supplies deionized water from the water bottle to the needle wash station. In case this tubing is empty, please fill it first before starting measurements.

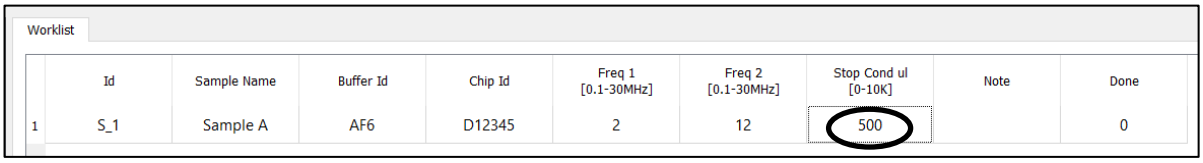
- Make sure that the autosampler water bottle is full of deionized water and that the lid of the peristaltic pump of the autosampler is closed
- Make sure that the autosampler is switched on and activated (*Online* status in the *Autosampler* section of the *Admin* tab in AmphaSoft)
- In the menu, select *Tools* > *Autosampler* > *Commands* > *Pump.* This command will start the pump for 30 seconds. Repeat this command until the tubing is supplying water to the needle wash station (central container). In case no water is supplied, make sure the lid of the autosampler peristaltic pump is closed and correctly adjusted (see also 7.11.5).

7.7 Setting up a Measurement Series

- Start the instrument and autosampler
- Connect to the instrument and activate the autosampler in the *Admin* tab of AmphaSoft
- Open the Worklist
- Configure the first measurement (sample name, chip ID, buffer…)
- Add a reasonable µl (microliter) stop condition in the column *Stop Cond Ul.* We recommend setting a time stop condition too, e.g. 25 s for G chips, 30 s for E chips and 1 min for D and F chips. In addition, a *cells* stop condition of 10’000 can be recommended for most applications. Please contact support@amphasys.com for questions about the experimental setup.

Note: **Stop Conditions**

Set the µl stop condition reasonably, such that no air is aspirated when the well is empty. Keep in mind that there is some dead volume in the well which the needle cannot reach.



Note: Measurement Sequence

In case you want the autosampler to perform a non-default sequence of measurements (default well sequence = A1 > A2 > A3…), you can specify the well for each sample. To do that, display the *Well Id* column in the Worklist. Use the *Settings +/-* button on the bottom of the Worklist and select *Well Id*. The *Well* column now displays the assigned well coordinates (default) in the format <Plate number><Row><Column>. As an example, "*2B04*" corresponds to the well B-4 on the second plate, i.e. sample *S_112*. When 2B04 is entered as a well coordinate for S_1, the sample S_1 (typically the first sample of the run) will be aspirated at position 2B04.

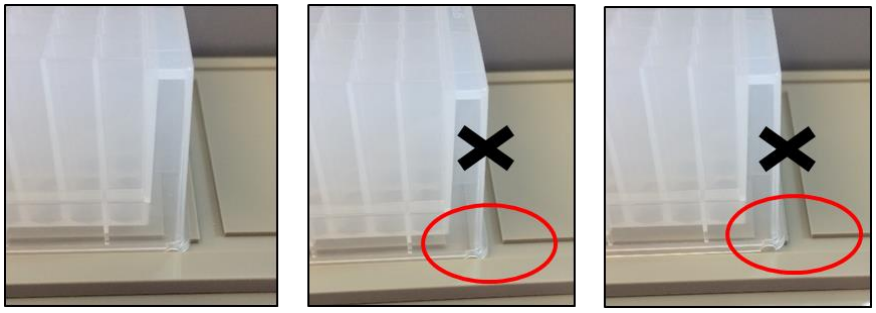
Note: High Throughput Measurements

If process time is an important criterium, the flush between individual measurements can be skipped. In that case, the flush will be replaced by an air bubble purge. It has been shown that this procedure has no adverse effects on carryover. The air bubble purge option can be selected by displaying the *Flush* column in the Worklist. Use the *Settings +/-* button on the bottom of the Worklist and select *Flush.* In the Flush column, replace the 1 (flush) by a 0 (air bubble purge).

Note: Optimized Resuspension Time

Depending on the pollen type used, the resuspension time can be adjusted. Quickly sedimenting or agglomerating particles require longer resuspension times than particles which stay well distributed over extended periods. A default resuspension time of 10 seconds is set. The resuspension time can be adjusted between 0 (no resuspension) and 60 seconds. Use the *Settings +/-* button on the bottom of the Worklist and select *Resuspension.* In the *Resuspension* column, enter the desired resuspension duration in seconds.

- Add more measurements if required
- Save the workspace (!)
- If not yet empty, empty Ampha Z32 and autosampler waste bottles
- Fill the Ampha Z32 water bottle (250 ml) and the autosampler water bottle (500 ml) with deionized water
- Perform a needle adjustment in case one of the conditions listed in section *7.5 Adjusting the Needle Position* applies.
- Place the measurement chip and perform an initial rinsing
- Prepare samples and pipet samples into a 96-well microtiter plate (2 ml capacity, maximum height = 44 mm, max. 1.8 ml sample / well)
- Place the 96-well microtiter plate correctly on the left position of the autosampler stage.



Attention Be very careful to place the plate correctly. Otherwise, the autosampler needle may crash into the plate.

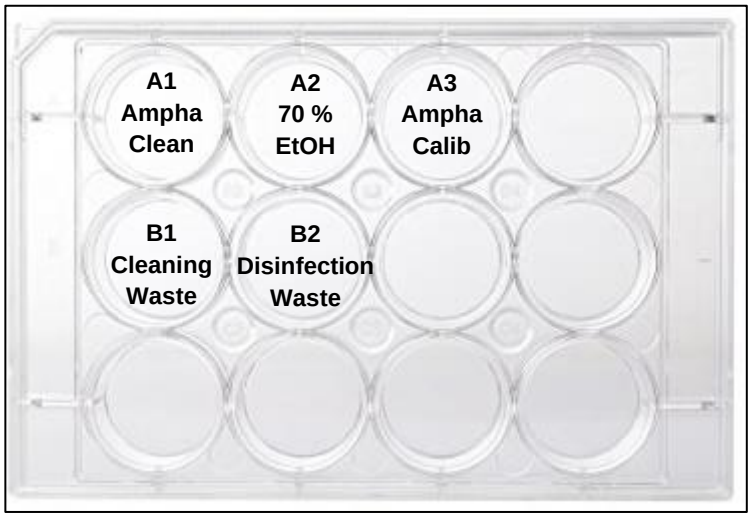
- Select the first measurement (typically S_1) in the Navigation Panel and click *Start Measurement*. This initiates the measurement series.

Attention If you intend to measure more than 96 samples, please check your waste and water containers during the run. The containers are large enough for a run of 96 samples.

Procedure of a measurement

7.8 Instrument Shutdown

After finishing the experiments, the Ampha Z32 and autosampler fluidics must be cleaned with AmphaClean solution. Depending on the application, an additional cleaning with 70 % ethanol is recommended (see also chapter *6 Maintenance*). These liquids are filled into a 12-well microtiter plate at dedicated positions (see loading scheme below).



Maintenance Loading Scheme

A dedicated 12-well microtiter plate is used for instrument maintenance and chip tests. The liquids for each test are pipetted in the corresponding well in the A-row of the 12-well plate. During the processes, waste is delivered into wells in the B-row.

The plate can be reused after thorough cleaning and drying.

7.8.1 Cleaning with AmphaClean

- Take a clean 12-well microtiter plate and fill 4 ml AmphaClean in well A1. Place the plate on the left plate position of the autosampler stage. Make sure the lid is not on the plate.
- In the *Basic* tab of *AmphaSoft*, check the *Cleaning* checkbox and click *Start Rinsing*.
- After finishing, empty the wells of the microtiter plate and clean the plate for re-use.
- Empty the Ampha Z32 and autosampler waste bottles.

7.8.2 Disinfection with 70 % Ethanol

- Take a clean 12-well microtiter plate and fill 5 ml 70 % ethanol in well A2. Place the plate on the left plate position of the autosampler stage. Make sure the lid is not on the plate.
- Configure a *Cleaning* chip in the dropdown menu of the *Measurement* tab.
- Insert a cleaning chip into the instrument and close the lid.
- In the *Basic* tab of *AmphaSoft*, check the *Disinfection* checkbox and click *Start Rinsing*.

Attention Only use a cleaning chip for instrument disinfection.

- After finishing, empty the wells of the microtiter plate and clean the plate for re-use.
- Empty the Ampha Z32 and autosampler waste bottles.

7.9 Chip Test

- Take a clean 12-well microtiter plate and fill 4 ml AmphaCalib in well A3. Place the plate on the left plate position of the autosampler stage. Make sure the lid is not on the plate.
- Make sure that you entered the Chip ID of the chip you want to test in the worklist. The chip ID must appear at least once in the worklist.
- In the main menu select *Tools* > *Chip Test*. Select the appropriate chip from the dropdown menu and click *Start*.
- After finishing, empty the well of the microtiter plate and clean the plate for re-use.
- Empty the Ampha Z32 waste bottle.

7.10 Maintenance

In case the needle wash station or the waste collector become dirty, clean them with deionized water and a cotton swab. After cleaning, rinse the inside of both compartments well with deionized water to remove any remaining fibers from the cotton swab. You can remove the water of the outer compartment of the water container by switching on the pump:

- Make sure that the autosampler is switched on and activated (*Online* status in the *Autosampler* section of the *Admin* tab)
- In the menu, select *Tools* > *Autosampler* > *Commands* > *Pump.* This command will start the pump for 30 seconds.

The peristaltic tubing and needle should be replaced at least annually. Please contact Amphasys for replacement material.

7.11 Troubleshooting

7.11.1 Autosampler arm gets stuck

In case the autosampler fails to move the needle during the initialization sequence, just restart the autosampler. If it fails again, switch the autosampler off and try to move the toothed rack which contains the needle carefully up and down. Then, switch on the autosampler. In case the problem persists, contact Amphasys customer support.

7.11.2 Clogging Detection Message

In case a clogging occurred, you will be notified with a popup window. If you by chance see that a clogging is about to occur (i.e. when the silicone tubing is expanding), you can also stop the current process, e.g. by clicking *Stop Measurement*.

When a clogging was detected, please follow the steps described below:

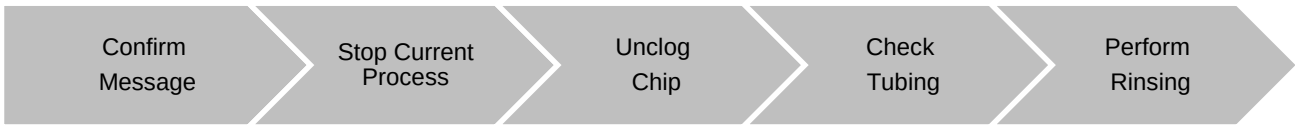
1. Confirm the notification

2. Stop the current process, i.e. click *Stop Measurement* or *Stop Rinsing*.
3. Remove the chip and unclog it using the wash station (see chapter *6.3.4 Using the Wash Station to Unclog Chips*). After unclogging, place it back in the instrument and close the lid.
4. Inspect the tubing system. If the silicone tubing jumped from the adapter, attach it again.
5. Perform an instrument rinsing: In the *Basic* tab, click *Start Rinsing*. In case the clogging message occurred due to a large air bubble in the system, the message can occur again during the rinsing. In that case just perform additional rinsings to fill the fluidic system (see also *7.11.3 Clogging Detection Message without Clogging*.

Once you have completed these steps, select the measurement where you want to resume your measurement series and click *Start Measurement*. The selected measurement will start as usual and the series will continue.

Note If you want to repeat the measurement in which the clogging occurred, please make sure that the remaining amount of liquid is sufficient. If not, dilute the sample with measurement buffer or prepare the sample again.

Procedure of resolving a chip clogging



7.11.3 Clogging Detection Message without Clogging

The clogging detection algorithm is designed to detect very low flow rates. This can be the case for a clogging or for a large air bubble.

Large air bubbles can be the case for the following reasons

- Fluidic lines empty after transport or maintenance. Perform an Ampha Z32 instrument rinsing to fill the fluidics. In case the clogging detection message occurs again (fluidics not filled yet), repeat the rinsing.
- Ampha Z32 water bottle empty: Air is pumped through the fluidic system during flushing, rinsing, cleaning or disinfection processes. Refill the water bottle.
- Sample empty: The µl stop condition was set too high, so that the sample emptied and air was aspirated. Please make sure that enough sample is available or adjust the stop conditions by setting time and cells stop conditions in addition.
- Leaky fluidic system: Try to localize the defect (adapters, screws…) and contact Amphasys customer support.

In all cases, perform an instrument rinsing after resolving the problem. It is possible that during the rinsing another clogging detection message will appear, because the tubing still contains air. In that case, just confirm the message, stop the rinsing and start it again. Repeat this procedure until a rinsing can be performed successfully without the clogging detection notification.

7.11.4 Resuming Measurements

Measurement series can be interrupted due to several reasons:

- Manual interruption (clicking stop measurement during loading or measuring)
- Clogging detection
- Laptop in sleep mode

In all cases, it is possible to resume the measurement series.

- For manual interruptions and clogging detection (after resolving the clogging), just select the desired measurement in the *Navigation* panel and click *Start Measurement* in the *Measurement* tab. The measurement series will continue again, starting from the selected measurement.
- For interruption due to sleep mode, please restart both instruments (Ampha Z32 and autosampler), reopen AmphaSoft and reconnect to the Ampha Z32 and activate the autosampler. Load the workspace and perform an initial rinsing before resuming the measurement series. The measurement series can be resumed by selecting the desired measurement in the *Navigation* panel and clicking *Start Measurement* in the *Measurement* view.

7.11.5 No Liquid Supplied to Needle Wash Station

In case no liquid is supplied to the needle wash station or if no liquid is removed from the waste collector, please check the tubings for damage. In case the tubings are fine, check that the lid of the autosampler peristaltic pump is fastened and that the lid is pressing on the tubings. If the lid is loose, adjust the screw of the latch that is fastening the lid.

For testing purposes, you can start the pump in AmphaSoft:

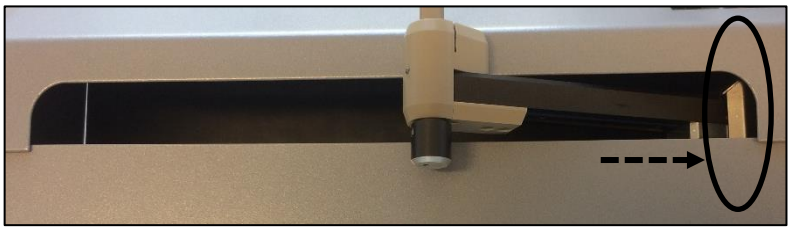
- Make sure that the autosampler is switched on and activated (*Online* status in the *Autosampler* section of the *Admin* tab)
- In the menu, select *Tools > Autosampler > Commands > Pump.* This command will start the pump for 30 seconds. During these 30 seconds you can adjust the screw. Tightening the screw too strong will result in an audible change of the sound of the pump motor. Tightening not strong enough will result in no flow.

7.11.6 Crash

In case the autosampler needle crashes, please immediately switch off the device and remove the plates from the autosampler stage. Then, restart the instrument and let it perform the initialization sequence. If the needle is damaged, do not use it anymore and immediately contact Amphasys.

7.12 Transportation and Shipping

- Unplug all cables
- Remove the autosampler needle and the corresponding tubings
- Carefully slide the autosampler arm to the very right position



- Important! Make sure the autosampler arm cannot move during transportation. Use appropriate packaging materials and adhesive tape to fix it completely.

Important Make sure the autosampler arm cannot move during transportation. Use appropriate packaging materials and adhesive tape to fix it completely.

- Carefully pack the instrument and accessories

8 Tips & Tricks

8.1 Ampha Z32 Settings and Chip Selection for Pollen Measurements

For the measurement of pollen we provide species-specific templates, which can be downloaded from our website.

Default frequency settings in AmphaSoft 2.0 are 2 MHz and 12 MHz. Standard gain settings are: Modulation - 3, Amplification – 6 and Demodulation - 2. The triggering level is set to 0.1 V and x positive is selected as triggering source and direction. Measurement settings can be optimized in the *Worklist* or in the *Advanced* tab.

The following table contains recommended chip types and filter types for different pollen sizes.

Pollen size	Chip type	Filter mesh size
5 - 20 µm	C chip – 50 µm	30 µm
10 – 40 µm	F chip – 80 µm	50 µm
25 – 70 µm	D chip – 120 µm	50 or 100 µm
50 – 130 µm	E chip – 250 µm	100 or 150 µm
100 – 250 µm	G chip – 400 µm	200 or 300 µm

For further explanations, please refer to the *AmphaSoft 2.0 User Guide*, the *Quick Guides* and the Amphasys website.

Please use our pollen list to get information about pollen size and recommended buffers. It can be downloaded from our website. https://amphasys.com/amphasys-downloads/#product-downloads

In case you are setting up an experiment with pollen not mentioned in the pollen list, please contact support@amphasys.com.

The following links may also help you to determine the pollen size of other species:

http://pollen.tstebler.ch/MediaWiki/index.php?title=Pollenatlas&setlang=en

http://blogs.cornell.edu/pollengrains/all-species-represented/

https://www.polleninfo.org/DE/en/aerobiology/pollen-atlas.html

http://www.pollenflora.it/Accorsi-Foto-Polline/Contenuto.html

https://www.paldat.org/search/A

8.2 Measurement Buffer Selection

When setting up new measurements, please consult our *Quick Guides*, the *Pollen Analysis Instructions* and the Template section on our website. They contain valuable information, such as recommended buffers, filters, chips and species-specific configurations of the instrument.

https://amphasys.com/amphasys-downloads/#product-downloads

8.3 Sample Preparation

Sample preparation methods vary depending on cell types and source. However, some general recommendations can be formulated.

- In case of dehydrated samples, rehydrate them properly before suspending in the buffer. Have a look at our *Pollen Rehydration Quick Guide*.
- After suspending, mixing and filtration, measure the cells directly. At extended incubation times, cells may die or clump.
- Use always dry filters. Residual water in the filter changes the conductivity of the sample.
- Make pollen dead-controls either by one of the following methods (select the one that works best)
    - Heating pollen samples in a small amount of buffer about 15 minutes at 70 ºC in a closed Eppendorf tube, then cool down the sample to ambient temperature, filter, dilute and measure.
    - Heat pollen samples (not suspended in buffer) in a closed Eppendorf tube for 30 minutes in boiling water, then add pollen to buffer, suspend, filter and dilute before measuring
    - Heat pollen samples in a microwave
    - Use old pollen as a dead-control or leave the pollen several days at room temperature (species dependent).
- It is generally recommended to filter pollen after heat-treatment to remove clumps.

More information can be found in the *Sample Preparation Quick Guide*.

8.4 Temperature

Variations in the operating temperature of the instrument and the temperature of the buffer can result in phase shifts. Please take temperature-dependent phase shifts into consideration when planning experiment series that last over extended periods (e.g. measuring outdoors from early in the morning to late in the afternoon).

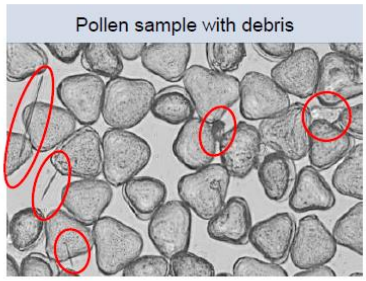
For optimal comparability of results, we recommend performing measurement series at stable conditions.

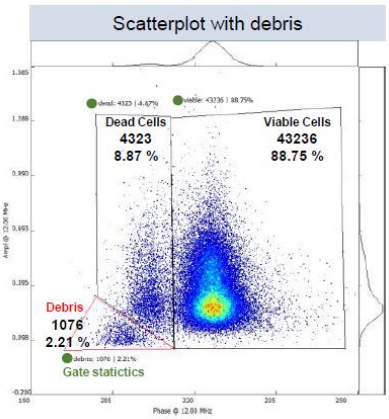
8.5 Debris

8.5.1 What is debris?

Depending on the sample preparation method and the instrument settings, a flow cytometric scatterplot does not only show the cells of interest, but also other particles. These particles can be cellular fragments or other material, commonly classified as *debris*.

In impedance flow cytometry scatterplots, such debris events are located at the bottom of the plot, i.e. at low amplitudes. Importantly, debris signals result from pulse pairs that were classified as true events. Therefore, they also go into the measurement statistics (i.e. number of accepted cells and concentration). In order to obtain correct viability information, the occurrence of debris must be prevented or corrected.





8.5.2 How to deal with debris

1. Improve sample preparation

Prepare cell suspensions as pure as possible. Have a look at the *Sample Preparation Quick Guide*.

2. Use the *Hide Cells* feature

This software feature can be used to mark datapoints as debris using a polygon gate. Once hidden, those datapoints are not used for gate statistics anymore. More about this feature is shown in the *AmphaSoft 2.0 User Guide*.

3. Increase the level to exclude debris (advanced)

Debris-events typically have lower signal amplitudes than the cells of interest. Therefore, increasing the level setting leads to the exclusion of debris, as these signals are not recognized as true events anymore. Please only adjust the level settings if you are an advanced user and if you know the signal properties of all your cells of interest (dead and viable cells; oscilloscope view).

9 Warranty, Technical Support and Ordering

9.1 Limited Warranties

Limited warranty period is one (1) year from first operation of the product by buyer. It covers PC software and embedded software, and defects in material and workmanship under normal use and does not apply to ordinary wear and tear. For detailed information, refer to the warranty information at https://amphasys.com/.

9.2 Extended Warranties

Extended warranties are available. Visit https://amphasys.com/ for information.

9.3 Technical Support

For technical support, please contact

Amphasys AG

Technopark Lucerne

Platz 4

CH-6039 Root D4, Switzerland

Phone: +41 41 541 91 20

Email: support@amphasys.com

9.4 Ordering Information

For a complete list of parts and reagents, please visit https://amphasys.com/ampha-z32-pollen-analyzer/#consumables .