Ampha™ Z30

IMPEDANCE FLOW CYTOMETER

USER MANUAL

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Content

1 Introduction

Ampha Z30 takes advantage of the superior sensitivity of its microfluidic chip for measuring the electrical properties of any kind of cells (yeasts, plant, pollen, bacteria, animal and human cells). This technology does not need the use of specific biomarkers, thus, most analyses can start right after sampling. The device can measure changes in cell size, membrane capacitance and cytoplasmic conductivity, parameters whose alterations characterise 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 and ploidy analyses.

1.1 About the User Manual

This User Manual provides detailed information about the Ampha Z30 impedance flow cytometer setup, requirements, specifications, and maintenance, including troubleshooting help and service information. Information about AmphaSoft is supplied in the separate *AmphaSoft SW User Guide*. Further details, accessories, chips, etc. you can find at www.amphasys.com/community .

Figure 1: The Ampha Z30 Impedance Flow Cytometer System

1.2 Delivery and Inspection

The Ampha Z30 system includes AmphaSoft for controlling the instrument, data acquisition, and data analysis, which runs on a Windows-based laptop PC with an USB-dongle for the software license.

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 Specifications

Impedance Chip	
Channel dimensions	15 x 15 µm / 30 x 30 µm / 50 x 50 µm / 120 x 120 µm / 250 x 250 µm or other types on request depending on application
Impedance measurement	
Frequency range	100 kHz ï 30 MHz
Frequency selection	Up to 4 different frequencies simultaneously
Analysis range	
Sample volume	50 ï 2000 µl
Concentration range	1 x 10^3 to 1 x 10^7 cells /ml
Particle size	1 - 60 µm (up to 150 µm with 250 µm chip)
Fluidics	
Sample flow rate	5 ï 2500 µl/min, dependent on chip
Pump	Peristaltic pump with disposable pump head
Labware compatibility	5 ml polystyrene round-bottom tubes (Falcon® PP 352002/PS 352003/ Sarstedt PP 55.1579)
Operating System	Windows 7 or 8
Software	AmphaSoft, data in fcs3-format available
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. 3A, < 90 W

Table 1: Specifications of Ampha Z30

2 Hazard and Precaution

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. Advices given in this handbook are intended to supplement, not supersede, the normal safety requirements prevailing in the userôs 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.

2.1 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:

- Å Make sure the line power cord is connected to a line power outlet that has a protective conductor (earth/ ground).
- Å 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 unfavourable 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 (arrows in Fig. 2). The instrument is supplied with a 90W industrial adaptor power supply, which provides output voltages of max. 24V. 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 an incorrect functioning of the instrument or even to its damage.

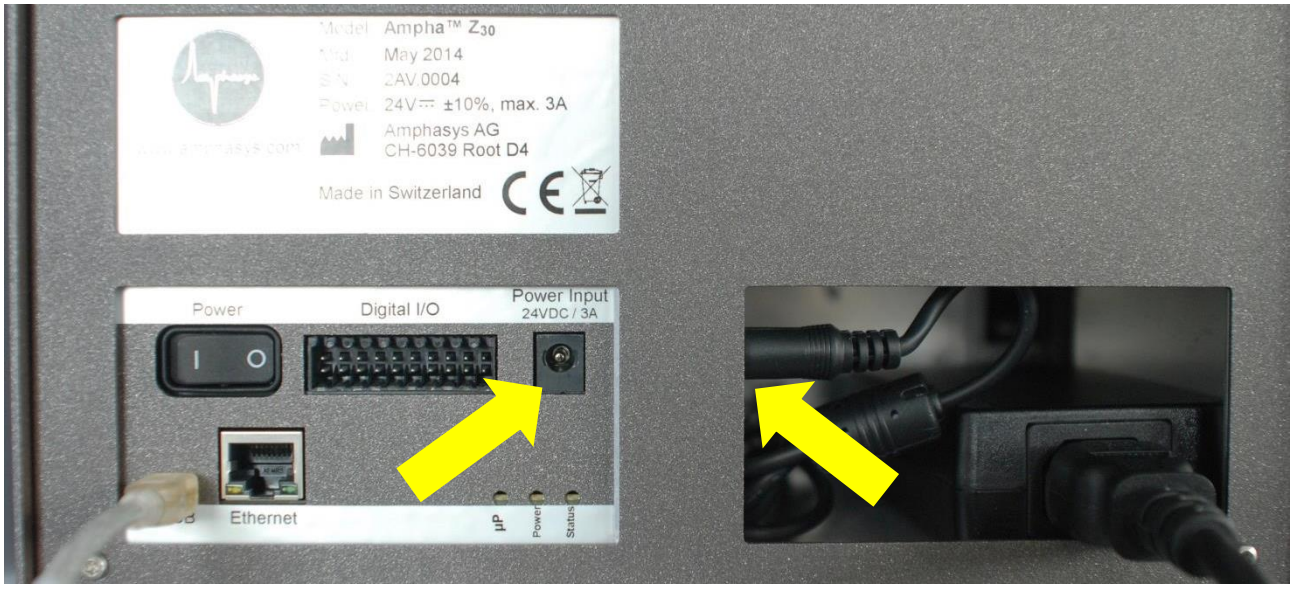


Figure 2: Ampha Z30 rear side. Arrows indicate the plugs for the connection of the power supply.

2.2 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 of properly in accordance with the local safety regulations. Refer to your local safety regulations for proper disposal procedures.

2.3 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.

3 Setting up Ampha Z30

3.1 Installation

The instrument and the associated laptop PC are normally installed by Amphasys including some general instructions and training.

3.1.1 Computer

Installation and operation of the instrument Ampha Z30 requires a separate laptop PC, which is usually configured and supplied by Amphasys. The PC should be connected with the supplied power cord to an AC power outlet 110-240V AC, 50/60Hz.

3.1.2 Software and Dongle

The instrument is operated with the software AmphaSoft, which is preinstalled with Windows™ 7 on the PC. Update versions will be provided by Amphasys or can be downloaded from Amphasysô website in the download area.

The use of AmphaSoft needs a license, which is provided with a USB-dongle. This dongle has always to be plugged in the PC or laptop to be used with the software or instrument. Further details are described in the *AmphaSoft user guide*.

3.1.3 Instrument

The instrument must be connected to an AC power outlet 110-240V AC, 50/60Hz. The power lines to the equipment should be voltage regulated and surge protected. Connect the instrument with the provided mini USB-cable to a USB-port of the PC (Fig. 2).

! Do not touch the gold contact pins in the lid and at the chip clamping system. For contacting problems contact Amphasys.

! Always unplug all cables before moving the instrument or laptop to other tables etc.

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.

3.1.4 AmphaChips

The AmphaChips listed in table 2 are the actual available chips from Amphasys. In the table 2 is also listed with which tubing set the chips can be applied and the recommended pump speeds for running. Recommended particle concentration and sample filtration is given in table 3, section 3.2.2 .

Chip specification	Chip channel diameter	Fluidic set	Pump range	Measurement pump speed
A00000	15 x 15 µm	S	1 ï 50 rpm	10 ï 20rpm
B00000	30 x 30 µm	M	1 ï 300 rpm	30 ï 50 µm
C00000	50 x 50 µm	M	1 ï 300 rpm	80 (50 ï 100) µm
D00000	120 x 120 µm	L	1 ï 300 rpm	60 (50 ï 100) rpm
E00000	250 x 250 µm	L	1 ï 500 rpm	300 ï 350 rpm

Table 3: AmphaChips, settings for fluidic set up.

3.2 Measurement

All information about AmphaSoft is supplied in the separate *AmphaSoft SW User Guide*.

3.2.1 Start

- ! Check liquid bottles: The front bottle normally contains deionized water, the bottle at the side is for waste liquid and should be possibly empty at the beginning of a test series. !

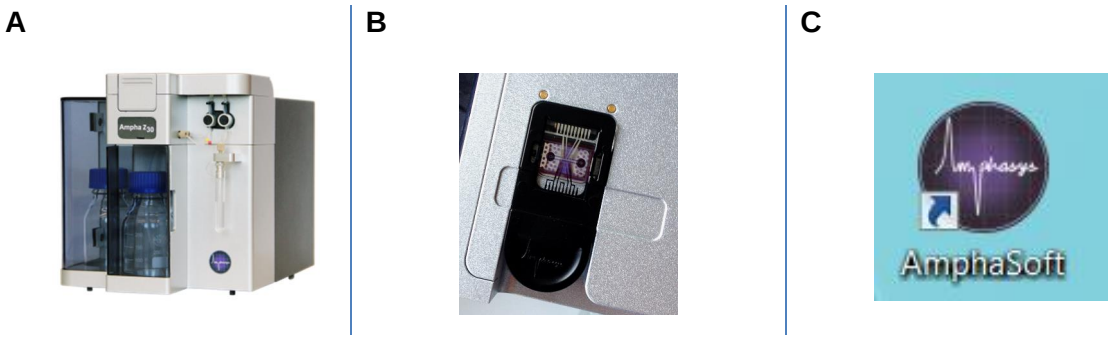


Figure 3: (A) Instrument, (B) Interface with chip, (C) Software shortcut icon on desktop

- Switch on the instrument, the power switch is located at the back of the instrument. Turn on the laptop PC. Make sure that the USB cable is correctly connected to your device and the USB-dongle with the AmphaSoft software license is plugged in.

- Start the software ñAmphaSoftò by double clicking the icon (Fig. 3C).

- Select your user mode, ñOnlineò if you are connected to the instrument and plan to perform a measurement, or ñOfflineò, if you want to load and analyse data obtained from a former experiment (Fig. 4).

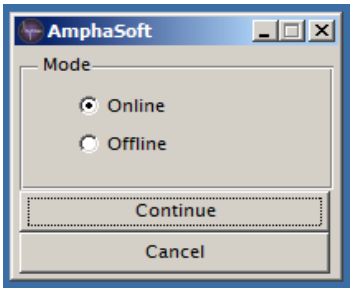


Figure 4: Selection of online or offline user mode.

- AmphaSoft is started with a Main GUI (Fig. 5). All information how to use or measure with AmphaSoft are explained in the ***AmphaSoft SW User Guide***.

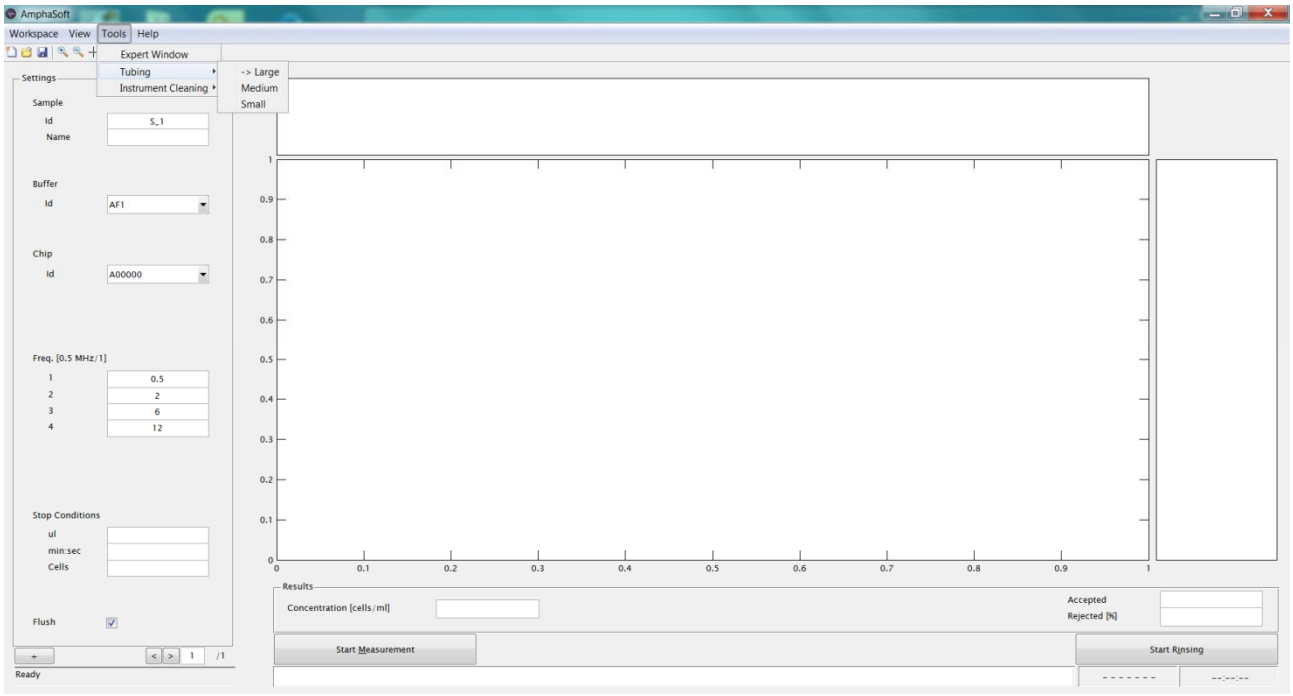


Figure 5: AmphaSoft Main GUI.

- **Sample preparation**: Make a single cell suspension in the correct buffer and filter the sample with an appropriate filter. Cell dilution is dependent on chip channel diameter (Tab. 4).

Chip channel diameter	Recommended cell concentration	Filter pore size
15 x 15 µm	1 ï 40 Mio per ml	5 or 10 µm
30 x 30 µm	0.5 ï 10 Mio per ml	20 µm (30 µm)
50 x 50 µm	0.25 ï 4 Mio per ml	30 ï 40 µm
120 x 120 µm	1000 ï 2000000 per ml	50 - 70 µm (100 µm)
250 x 250 µm	1000 ï 500000 per ml	150 µm (200 µm)

Table 4: Available AmphaChips, recommended cell concentrations for measurement and prefiltering.

4 Maintenance

! The instrument cleaning cycle should be performed as a part of daily measurement routine after the last measurement with AmphaClean solution as a cleaning detergent (Menu>Tools>Instrument Cleaning).
Keep tubings clean to avoid contamination with microorganisms. To prevent microorganism growth you can add Sodium Azide (NaN_3) to the water bottle at concentrations of 0.01% or less (**! toxic** compound!). Change the deionized water in the bottle regularly, especially before longer disuse of the instrument.
Empty the waste bottle regularly and clean it, best each day to prevent growth of microorganisms from back into the instrument.

In order to prevent system failures due to clogging, porous tubings or other problems with the fluidic system it is necessary to exchange the peristaltic pump head, including the tubings, the fluidic bottle filter and O-rings at least every two to three months. Shorter replacement cycles may be necessary if the instrument is heavily used. Omitting the replacement of the fluidics set may cause limited warranty.

The following table lists the parts and reagents that are required for regular maintenance (tab. 4). More details are available at www.amphasys.com/community.

Part Number	Description
12.100	Fluidic Set L for 120/250 µm chips
12.030	Fluidic Set M for 30/50 µm chips
12.015	Fluidic Set S for 15 µm chips
19.012-S	Sample Tubing S
19.012-L	Sample Tubing L
21.900	AmphaClean (Cleaning buffer)
11.901	Chip Wash Station

Table 4: Accessories and replacement parts for maintenance.

4.1 Change and Replacement of Fluidic Set

The Fluidic Set (see Fig. 6) is supplied with different tubing dimensions (S, M, or L), depending on the applications you are running on your device. The Fluidic Set was optimized and the Silicon tubings and fittings have been adapted (Fig. 6).

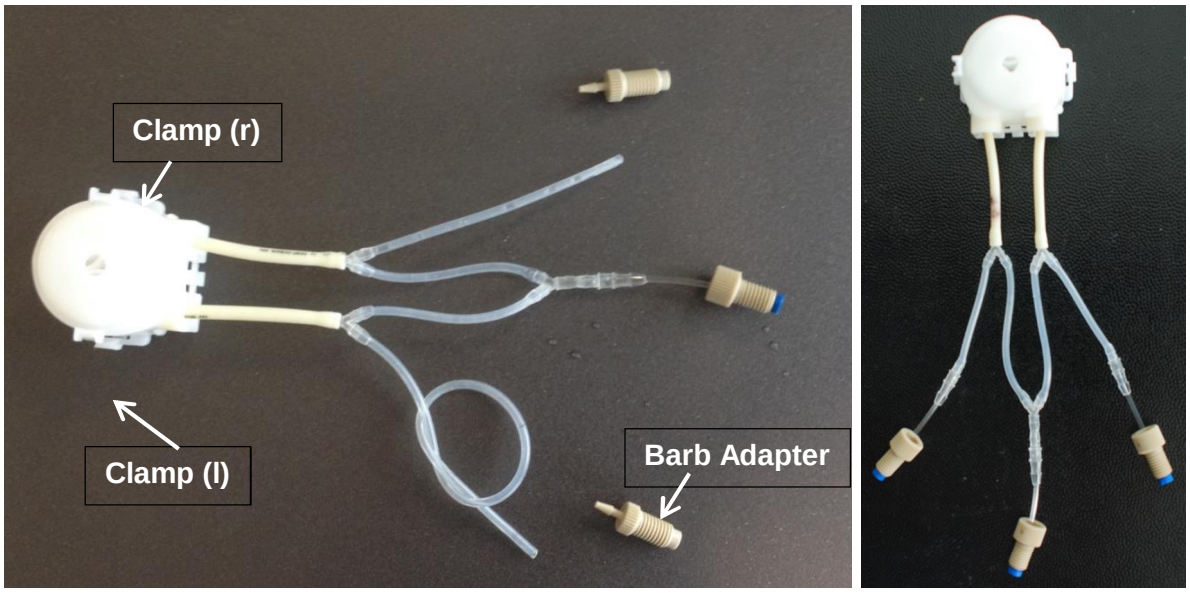


Figure 6: Fluidic Set including pump head left *NEW*, right *OLD* set.

4.1.1 Change from *OLD* to *NEW* Tubing Set

! With the change of the fluidic sets from old to new set, there is also a change of the Ampha Z30 internal fluidic by removing the check valve. For details you will be contacted by Amphasys or please contact Amphasys. !

Before removing the old tubing set, make sure that the peristaltic pump is not running by checking the software or looking at the pump head.

For changing the tubing set unscrew the three peek-fittings (Fig. 8A) from the old set and remove all tubings from the valves. Take out the pump head by pressing the left and right clamp and lifting the head (Fig. 6 and 7C). Place new tubing set with the pump head on the pump axis and press down that the clamps snap into place (Fig. 7C).

Screw in the two new *Barb Adapters* (Fig. 6, 7 A/B). Press both silicone tubings, ending in a Y-connection, into the inner valve ports (back) and the two silicone tubings with direct connection into the outer valve port (front, Fig. 7C, E, H). Screw the middle fitting (coming from Y-connector) in the outer port of the instrument (Fig. 7F). To screw the fitting into the corresponding port, check that the blue ferrule is flush with the tubing (Fig. 7D inlet). The peek nut should be loose at the tubing. Insert the tubing with the ferrule in place into the receiving port and while holding the tubing down firmly into the port, tighten the nut finger tight.

Push now the right silicone tubing on the barb adapter above the tube holder (Fig. 7F) and push the left longer silicon tubing on the barb adapter in the instrument wall (Fig. 7G). Be sure that the left longer silicone tubing has an easy loose knot, not pressing or squeezing the silicone tubing (Fig. 7E, H). Place this tubing as in Figure 7H, don't let it hang down.

Start the instrument and perform 2 to 3 times the rinsing program to fill the lines.

Figure 7: Change of new peristaltic tubing set including pump head.

4.1.2 Replacement of New Tubing Set

Before removing the old tubing set, make sure that the peristaltic pump is not running by checking the software or looking at the pump head.

For changing the tubing set unscrew the peek-fitting (Fig. 7D), drag down the both silicon tubings from the barb adapters (Fig. 7F, D) and remove all tubings from the valves. Take out the pump head by pressing the left and right clamp and lifting the head (Fig. 6 and 7C). Place new tubing set with the pump head on the pump axis and press down that the clamps snap into place (Fig. 7C).

Press the both silicone tubings, ending in a Y-connection, in the inner valve ports and the both silicone tubings with direct connection in the outer valve port (Fig. 7C, E, H). Screw the middle fitting (coming from Y-connector) in the outer port of the instrument (Fig. 7F). To screw the fitting into the corresponding port, check that the blue ferrule is flush with the tubing (Fig. 7D inlet). The

peek nut should be loose at the tubing. Insert the tubing with the ferrule in place into the receiving port and while holding the tubing down firmly into the port, tighten the nut finger tight.
Push now the right silicone tubing on the barb adapter above the tube holder (Fig. 7F) and push the left longer silicon tubing on the barb adapter in the instrument wall (Fig. 7G). Be sure that the left longer silicone tubing has an easy loose knot, not pressing or squeezing the silicone tubing (Fig. 7E, H). Place this tubing as in Figure 7H, don't let it hang down.
Start the instrument and perform 2 to 3 times the rinsing program to fill the lines.

4.1.3 Replacement of Old Tubing Set

Before removing the old tubing set, make sure that the peristaltic pump is not running by checking the software or looking at the pump head.
For changing the tubing set unscrew the three peek-fittings (Fig. 8A), remove the tubings from the valves. Take out the pump head by pressing the left and right clamp and lifting the head (Fig. 6 and 8B). Place new tubing set with the pump head on the pump axis and press down that the clamps snap into place (Fig. 8C).
To screw the fittings into the corresponding ports, check that the blue ferrule is flush with the tubing (Fig. 8D inlet). The peek nut should be loose at the tubing. Insert the tubing with the ferrule in place into the receiving port and while holding the tubing down firmly into the port, tighten the nut finger tight. Place the right fitting in the sample holder, the left fitting in the inner port (Fig. 8D). Press the both silicone tubings, ending in a Y-connection, in the inner valve ports and the both silicone tubings with direct connection in the outer valve port (Fig. 8E, F). Screw the middle fitting (coming from Y-connector) in the outer port of the instrument (Fig. 8F).
Start the instrument and perform 2 to 3 times the rinsing program to fill the lines.

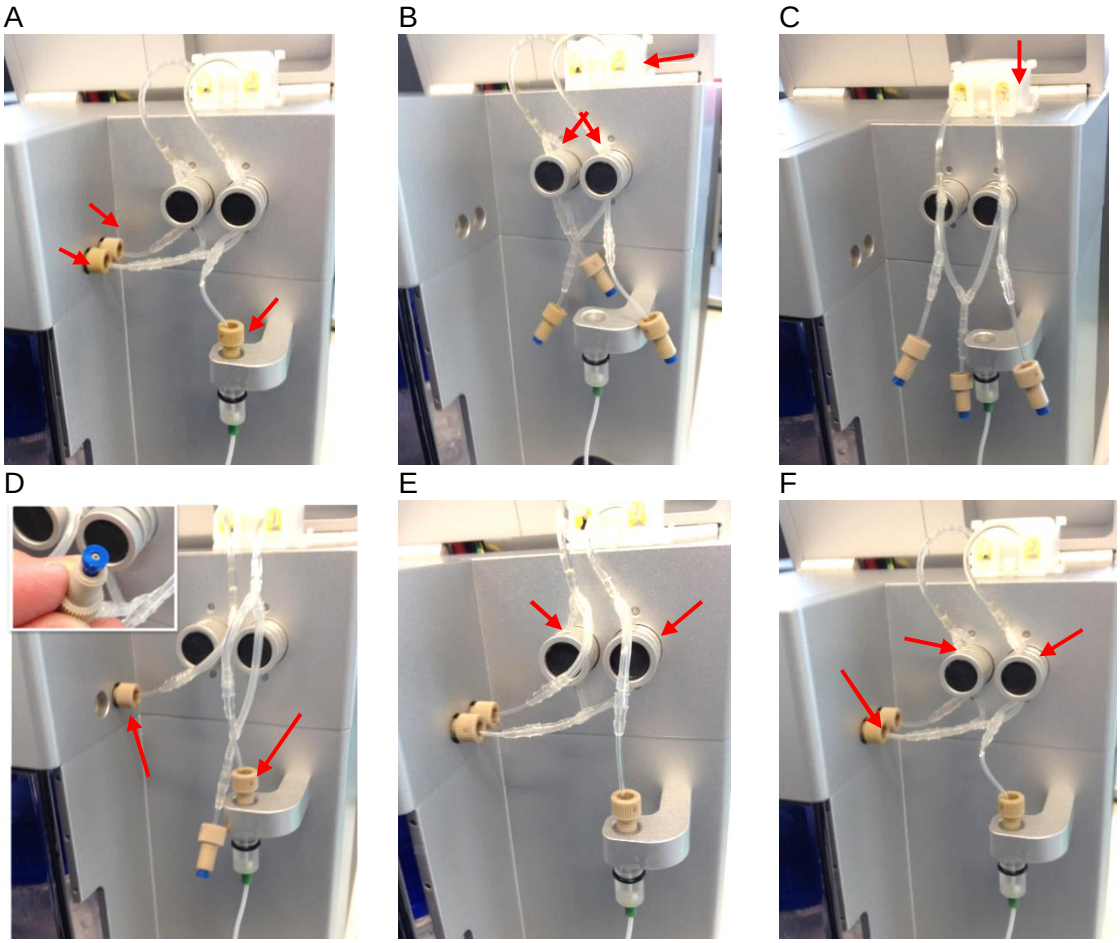


Figure 8: Change of old peristaltic tubing set including pump head.

4.2 Replacement of Water Bottle Filter

We recommend to replace the fluidic bottle filter (Fig. 9A) quarterly together with the fluidic set. For changing the bottle filter open the bottle and take it out from the instrument (Fig. 9B). Unscrew the filter from the plastic fitting (Fig. 9 C+D). Check that the tubing in the fitting has overhang of about 3 mm (Fig. 9D). Then finger tighten the fitting into the new filter (Fig. 9 B+E), place the filter back in the water filled bottle and close the lid. Perform 3 times the rinsing program to fill the filter and the tubing.

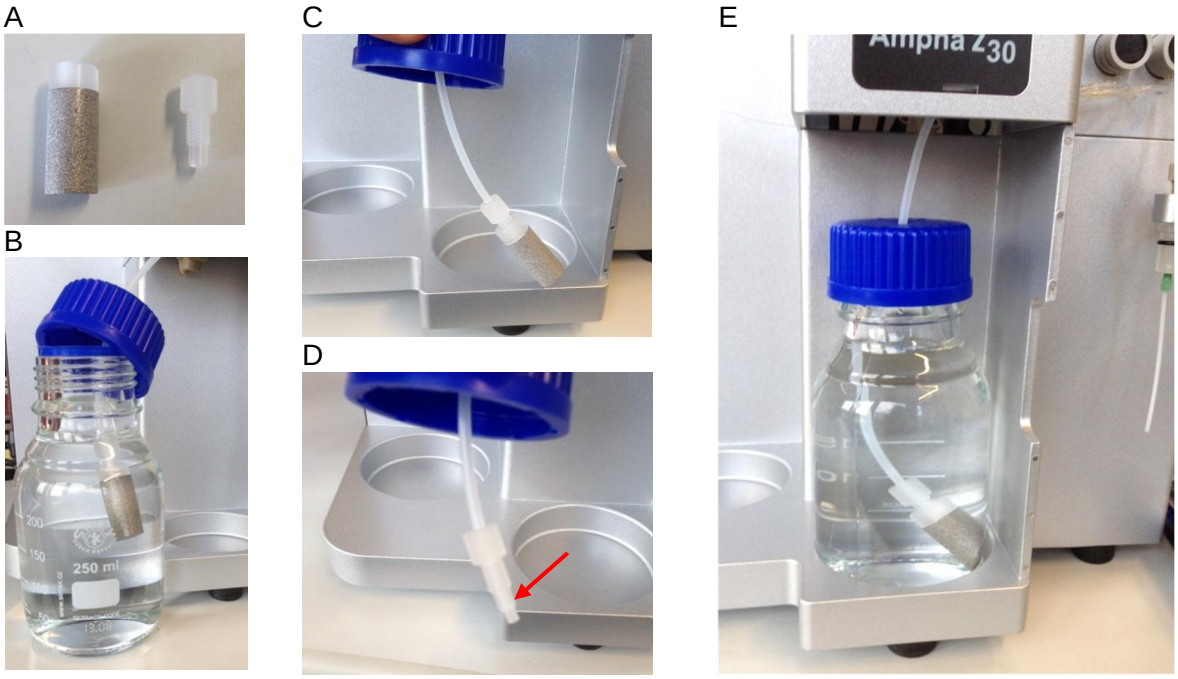


Figure 9: Change of fluidic bottle filter.

4.3 Fluidic Processes in Ampha Z30

There are several automatic running fluidic processes regulated by the software. The way the fluid moves, is explained below and shown in figure 10.

Rinsing / Flush: The left valve is switched and open in the back. The fluid runs first from the water bottle through the tubing in the back of the left valve and the pump head to the sample tube. Then you hear a click and both valves are switched left is open at front, right is open for back tubing. Fluid is running from water bottle through right valve and the pump towards the chip and waste. For **Rinsing** both processes run two times consecutive.

Measurement / Loading: Both valves are not active. The fluid is running from the sample tube through the right valve, the pump and the left valve to the chip and waste. Loading is with high speed of the pump and measurement with optimal speed for signal recording.

Cleaning: In the cleaning process for the daily shut down of the instrument first there is a rinsing cycle, followed by loading the AmphaClean through the chip to the waste. Afterwards there is running an additional rinsing cycle.

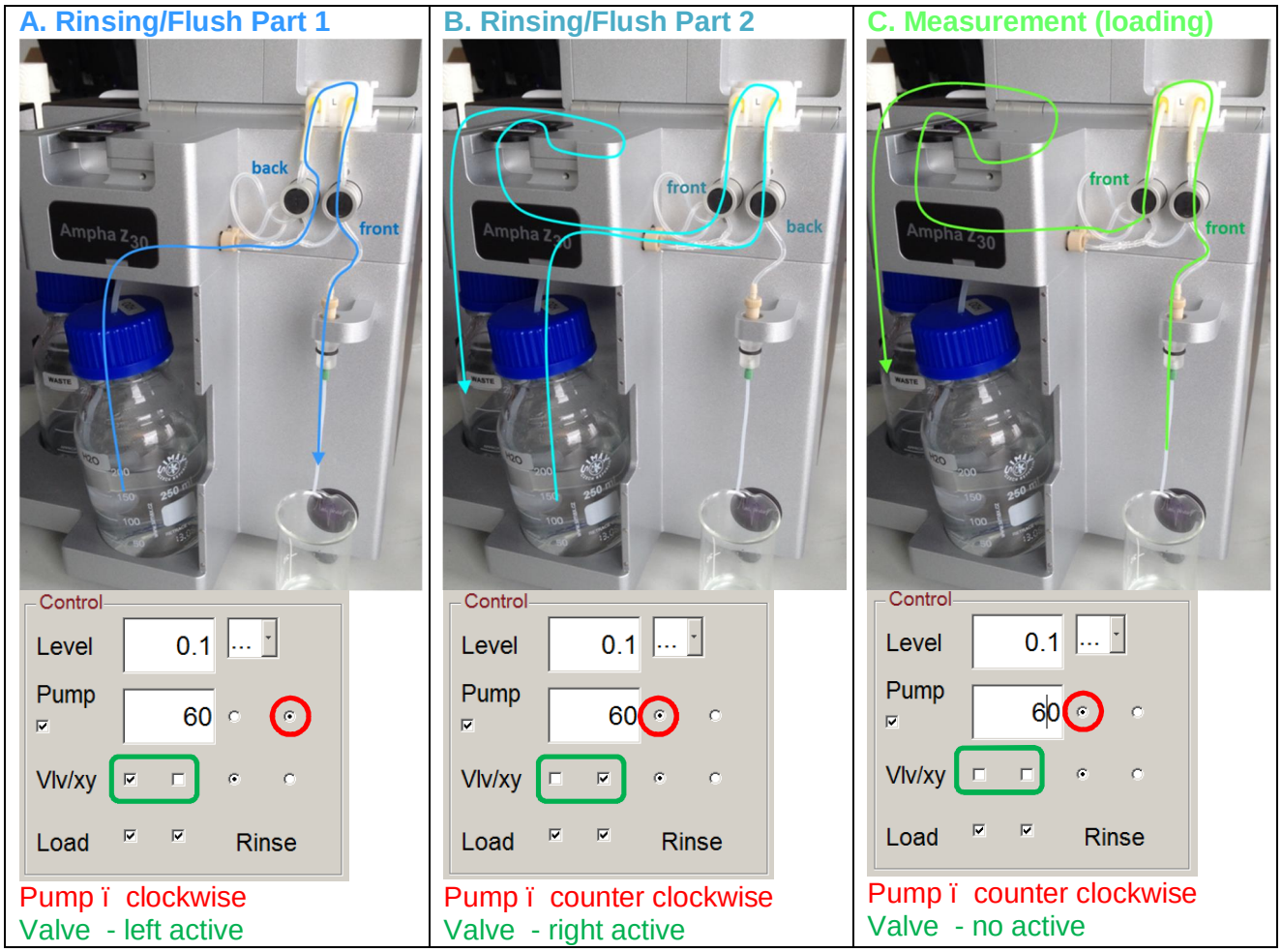


Figure 10: Picture with way of fluid on top, schematics of fluidic processes in Expert Window at the bottom.

When having problems with the fluidics, the Troubleshooting doesnô help; the fluidic processes can be regulated manually by using the expert window of AmphaSoft. The different possibilities are shown in Figure 11, but handle with care and contact Amphasys if you are not confident with functions.

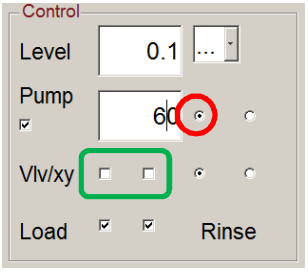
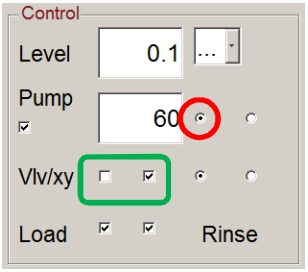
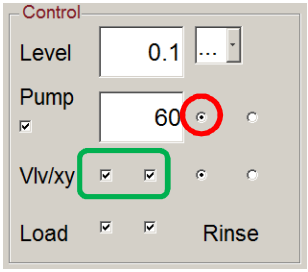
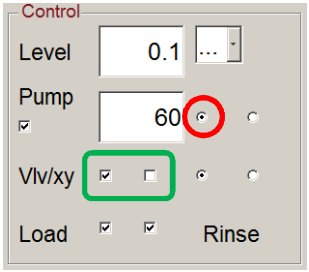
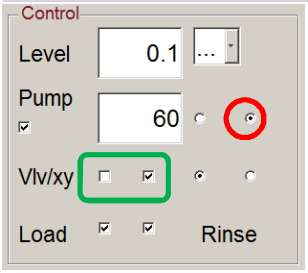
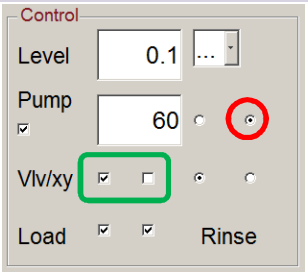
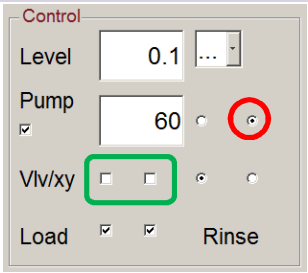
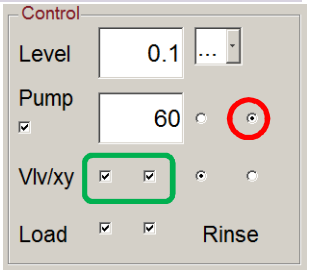
			
Sample to Chip	**System liquid to Chip**	**System liquid circular**	**Sample to system liquid**
Modes: Sample loading Measurement	**Modes:** Rinsing/flushing chip tubing Cleaning system	**Modes:** Not reasonable	**Modes:** Not reasonable
			
Chip (waste) to system liquid	**System liquid to sample**	**Chip (waste) to sample**	**Sample to system liquid**
Modes: Not reasonable	**Modes:** Rinsing/flushing sample tubing Cleaning sample tubing	**Modes:** Not reasonable	**Modes:** Not reasonable

Figure 11: Regulation of fluidic processes in Expert Window.

4.4 Chip cleaning

Chip insertion in chip holder:

- Keep the chip holder ready with the Amphasys sign facing upside.
- Take the chip with the holes facing down (the Amphasys logo is in the lower right side).
- Put the chip into the holder by carefully pressing the lateral clip until the chip is snapping in its place (Fig. 12)

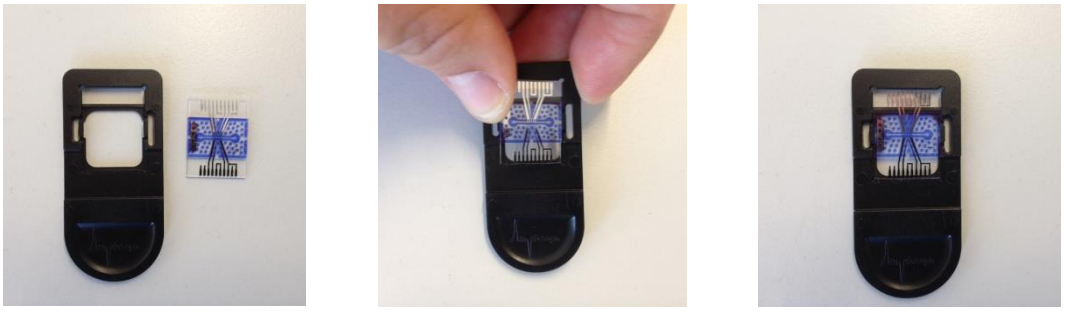


Figure 12: Placing the chip into chip holder.

Chip cleaning and storing:

- The chips are delicate microfluidic devices. They should be handled with care, cleaned regularly und stored in a dry and dust free box in the dark.
- The chip surface should be cleaned with a non-fuzzing tissue (e.g. Anticon® wipes) and water, if needed. Use of fuzzing tissue may cause clogging of the microfluidic channel.
- Chips should be cleaned immediately after use. There are flush, rinse and wash cycles that can be used while the chip is inserted in the instrument (see AmphaSoft User Guide). Alternatively, the chips can be washed with filtered (<0.5 µm) high-purity water or mild detergents (see below) in the chip wash station (Fig. 13) using a syringe (luer-lock connection).
- When using the chip wash station, general chip cleaning of non-blocked chips should be done from the inlet hole to the outlet hole. For de-blocking of clogged chips see instructions below.
- **Warning: Do not apply high pressure and do not use liquids with pH<6 and pH>8, alcohols or any corrosive agents. Doing so can lead to the destruction of the chips.**

Figure 13: Chip Wash Station, (A) placing of chip, (B) rinsing from inlet hole, (C) rinsing from outlet hole.

How to de-block clogged chips:

- When chips are clogged by dust, cells or any other particles, we recommend the cleaning procedure with the chip wash station mostly starting from the outlet hole (Fig. 13).
- Cleaning should be started with <0.5 µm filtered high-purity water. If necessary, other detergents can be used (see Tab. 5). Don't rinse with any organic liquids like alcohols.
- Checking the chip under a microscope usually helps to identify the cause of the clogging. When there is a blocking between inlet and the sensing channel, rinse from outlet hole, when there is a blocking between outlet hole and sensing channel, rinse from inlet hole.
- After special cleaning procedures always rinse the chip with water from the inlet hole. Blow out the water by pumping air through the chip wash station.
- Detergents can also be warmed to about 40 to 50 degrees for better cleaning effects.

Detergent	Usage
<0.5 µm filtered high-purity **water**	• general cleaning liquid
<0.5 µm filtered 2% **Neutracon** or solutions with dishwashing liquids	• chip cleaning of persistent biological cell blockings or residues in channels, holes • detergents should be used for maximal 10 minutes, because electrodes could be eroded after longer exposure
<0.5 µm filtered AmphaClean	• chip cleaning of dirt and persistent biological cell blockings or residues in channels, holes

Table 5: Chip cleaning detergents

Additional tips for still blocked chips

- After cleaning with water, let the chip dry out overnight and try to clean again.
- Try with ultrasonic bath, take out the chip from the chip holder, put it for 1 minute in the bath (containing clean water), put it back in the chip holder and try to clean again.
- If you know what kind of dirt is blocking the channel, you may try to dissolve the dirt with an appropriate solution/detergent. Keep in mind that the chip, channel structure and electrodes can be destroyed by certain chemicals. In case of doubt please contact Amphasys.
- Cleaning of the chip holes and channel with water or air and gentle pressure can be generated by a normal plastic syringe with a small silicon tubing on the top. Pressing this at the hole and either soaking or pressing can remove dirt (Fig. 14).

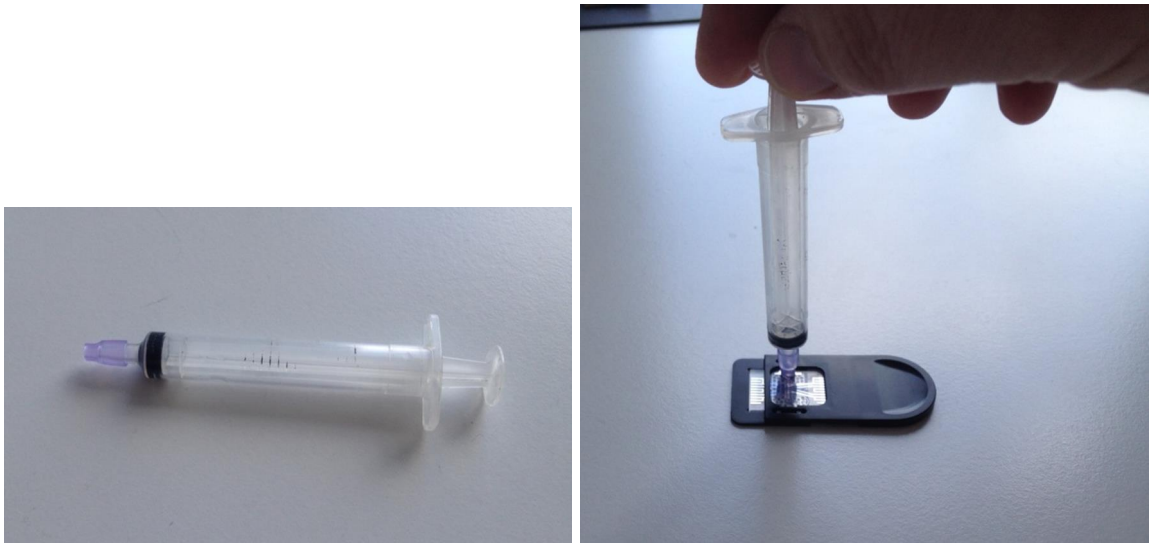


Figure 14: Syringe with silicone tubing for chip cleaning (left) and soaking at the inlet hole (right).

5 Troubleshooting and technical support

5.1 Troubleshooting

The tables (Tab. 6 and 7) contain the basic troubleshooting guidelines. If problems persist after performing troubleshooting, contact Amphasys Technical Support. Explanations and additional troubleshooting using the **Expert Window** in AmphaSoft are marked with **EW**. **Expert Window** is explained in detail in AmphaSoft User Guide.

When	Problem area	Problem	Cause	No in Tab 7
starting AmphaSoft software	General	the AmphaSoft program window does not appear OR appears only for some seconds	Instrument not switched on or not connected to the PC. Turn instrument on or connect USB-cable and restart AmphaSoft or Communication error	**A**
	Restart Error	Closing of AmphaSoft and immediately restart of the software ï error message	Communication error ï closing process not finished, wait always some seconds before restart AmphaSoft	**A3**
during any process		AmphaSoft doesnôt react, no response, any running process stops and does not progress	Communication error	**A**
during measurement		Measurement works only with low processing, high rejection rate or warnings/error messages of USB in log window	Communication error ï wrong data saving	**A4**
during fluid process		fluid process does not progress, AmphaSoft programme does not respond	Electronics error Communication error	**B** **A**
		fluid process stops abruptly	Electronics error Communication error	**B** **A**
No water	No liquid	No water is running during Rinsing in the sample tube or to waste, no flow detected	Fluidic problem	**D**
after sample loading	Signal	no signals are collected	Chip blocking Fluidic blocking Measurement with special buffers (Ficoll, sugars) Bad electronic connections Chip broken.	**C** **E** **F** **G** **C**
during measurement	Signal	signal collection stops after some time of measurement	Chip blocking Fluidic problem Measurement with special buffers (Ficoll, sugars) Chip broken.	**C** **D** **F** **C**
	Chip	If the problem with the Chip cannot be solved with any of the suggestions, put in another chip and try measurement.	Contact Amphasys	

Table 6: Problems with the system and during measurement.

No		Cause	Solution What to do	Success question	If yes, go to	If no, go to
A	**1**	Communication error	exit AmphaSoft and turn off instrument and PC, turn on instrument and PC and restart AmphaSoft	If exit of AmphaSoft is not possible with normal closing, stop process with TaskManager	problem solved	A2
	2		check USB connection from instrument to PC		A1	contact Amphasys

No		Cause	Solution What to do	Success question	If yes, go to	If no, go to
	3	Communication error - speed	Close the popup windows by confirming and press ESC, restart AmphaSoft		problem solved	A1
	4	Communication error ï low processing	During measurement save data only locally at the laptop, donôt use server connections for saving	Measurement works now with normal process speed and without any warnings or error messages	problem solved	contact Amphasys
B	**1**	Electronics error	exit AmphaSoft and restart it		problem solved	B2
	2	Electronics error	exit AmphaSoft and turn off instrument and PC, turn on instrument and PC and restart AmphaSoft		B1	contact Amphasys
C		Chip broken	Chip was tested , is broken or no solution found ï inform Amphasys			Use another chip, broken chips discard, for chips with problem contact Amphasys.
	1	Chip blocking	take out chip, put chip in rinsing device or look directly at it under the microscope	no blocking visible in front of the narrow channel?	C1.1	C1.2
	1.1		rinse the chip from the front hole with the syringe and (0.2 µm-filtered!) distilled water.	water comes out at the tube connected to the chip outlet hole?	problem solved	C1.2
	1.2		rinse the chip from the back hole with the syringe and (0.2um-filtered!) distilled water.	water comes out at the tube connected to the chip inlet hole?	C1	C2.1
	2.1		use other filtered liquids to rinse, from the back or from the front: mild detergent or alcohol (see Tab. 4)	liquid comes out at the tubes connected to the chip holes?	C1.1	C2.2
	2.2		fill chip with AmphaClean, incubate for 30 min maximum, rinse thoroughly with water	all cell-derived blockings are dispersed?	C1.1	C2.3, C2.4, C2.5
	2.3		wait until the chip channels are dry		C1.1	
	2.4		push air through the chip with the syringe		C1.1	
	2.5		fill the chip with water, put it in distilled water in an ultrasonic bath for 1 to 2 minutes		C1.1	Use another chip, for chips with problem contact Amphasys
		Attention: sample will be washed away during the following steps.				
D	**1**	Fluidic problem	check liquid bottles	Is water liquid bottle run dry?	fill the water bottle and run 3 times Rinsing	D2
	2		Water bottle is full, but tubing is empty?	Check if fitting to the bottle is fixed, Fig. 7, check connection of water filter in the bottle to the tubing Fig. 8 C/D, perform Rinsing	problem solved	D3
	3		Check if valves are functional, any dried sugar solutions within the valves from clogging problems can stop the switching	Clean the valves including the tubings outside with water, perform Rinsing and observe valve switching	problem solved	Try with section 4.3 or contact Amphasys for further instructions
E	**1**	Fluidic blocking	check liquid bottles	Is water liquid bottle run dry?	fill the water bottle and run 3 times rinsing	E2
		Use expert window				

No		Cause	Solution What to do	Success question	If yes, go to	If no, go to
EW	2		take out chip, put pump speed to 100 rpm, make sure to have liquid from sample loading, if not select right valve to switch to system water	Follow air gapes in the tubing to see liquid running; liquid comes out at the chip inlet O-ring?	problem solved, dry chip area, put in cleaned chip, uncheck valve and go on with your measurements	E3
EW	3		Check left valve, uncheck right valve, check pump to clockwise (right button) put pump speed to 100 rpm	Follow air gapes in the tubings to see liquid running; liquid comes out at sample loading	Yes, put valve and pump selection back E3.1	E4
EW	3.1		Unscrew fitting from tubing set to the chip (left, inner port), insert sample tube with water, uncheck valves, put pump speed to 100rpm or check right valve to use with system water	Liquid comes out at unscrewed fitting	E3.2	E4
EW	3.2		take out chip, unscrew a fitting with tubing from the cleaning device, screw it in the port to the chip, connect syringe to this tubing and try to push water	Water comes out at chip inlet O-ring?	Put all fittings back to their port, E2	Blocked check valve, exchange or cleaning necessary contact Amphasys
	4		Unscrew fitting from tubing set to the chip (left, inner port), insert tube with water, uncheck valves, put pump speed to 100rpm	Liquid comes out at unscrewed fitting	Yes, screw fitting, E3.2	E4.1
	4.1		Check tubing set for blocking, gaps etc.	Visible problems or breaks	new tubing set necessary	E4.2
	4.2		Unscrew all fittings from tubing set, rinse carefully with syringe from cleaning device from all fittings	Liquid drops at all sides	Yes, screw in system, E2	new tubing set necessary
F	1	Cell sedimentation	try to measure cells at higher speed, EW, adjust pump speed	EW, adjust pump speed, Cell sedimentation is reduced	problem solved, F2.1	F2.2
	2.1		sedimentation will appear further, use high concentrated samples and short measurement times, stay with higher pump speed			
	2.2		dissolve sample in more viscous liquid: prepare a solution with Ficoll400 (10-15%) in buffer (this slows down the fluidics, adjust pump speed in EW, rinse well afterwards)	Cell sedimentation is reduced to an acceptable level?	problem solved	contact Amphasys
G	1	Bad electronic connections	take out chip, try the following things:			
	1.1		dry electrical connections on chip and in the instrument with the special clean room towel provided	Measurements are okay?	problem solved	G1.2
	1.2		clean electrical connections on chip and in instrument with Acetone	Measurements are okay?	problem solved	G1.3
	1.3		Examine electrical spring contacts in the instrument to find out whether they are bent and do not contact the pads on the chip any more or whether they are even broken. Try to bend them carefully to a good chip contacting position.	Measurements are okay?	problem solved	G2

No	Cause	Solution What to do	Success question	If yes, go to	If no, go to
2		Put in another chip and try again.	Measurements are okay?	problem solved, The first chip was broken.	Try with another chip. If measurements are bad, contact Amphasys

Table 7: Possible solutions of problems listed in Tab. 6

5.2 Limited Warranty

Limited warranty period is one (1) year from first operation of the Product by Buyer. It covers software and firmware, 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 www.amphasys.com.

5.3 Extended Warranties

Extended warranties are available. Visit www.amphasys.com for information.

5.4 Technical Support

For technical support, contact:

Amphasys AG
Technopark Lucerne
Platz 4
CH-6039 Root D4, Switzerland
Phone: +41 41 541 91 20
Email: info@amphasys.com

5.5 Ordering Information

For a complete list of parts and reagents, please visit www.amphasys.com.