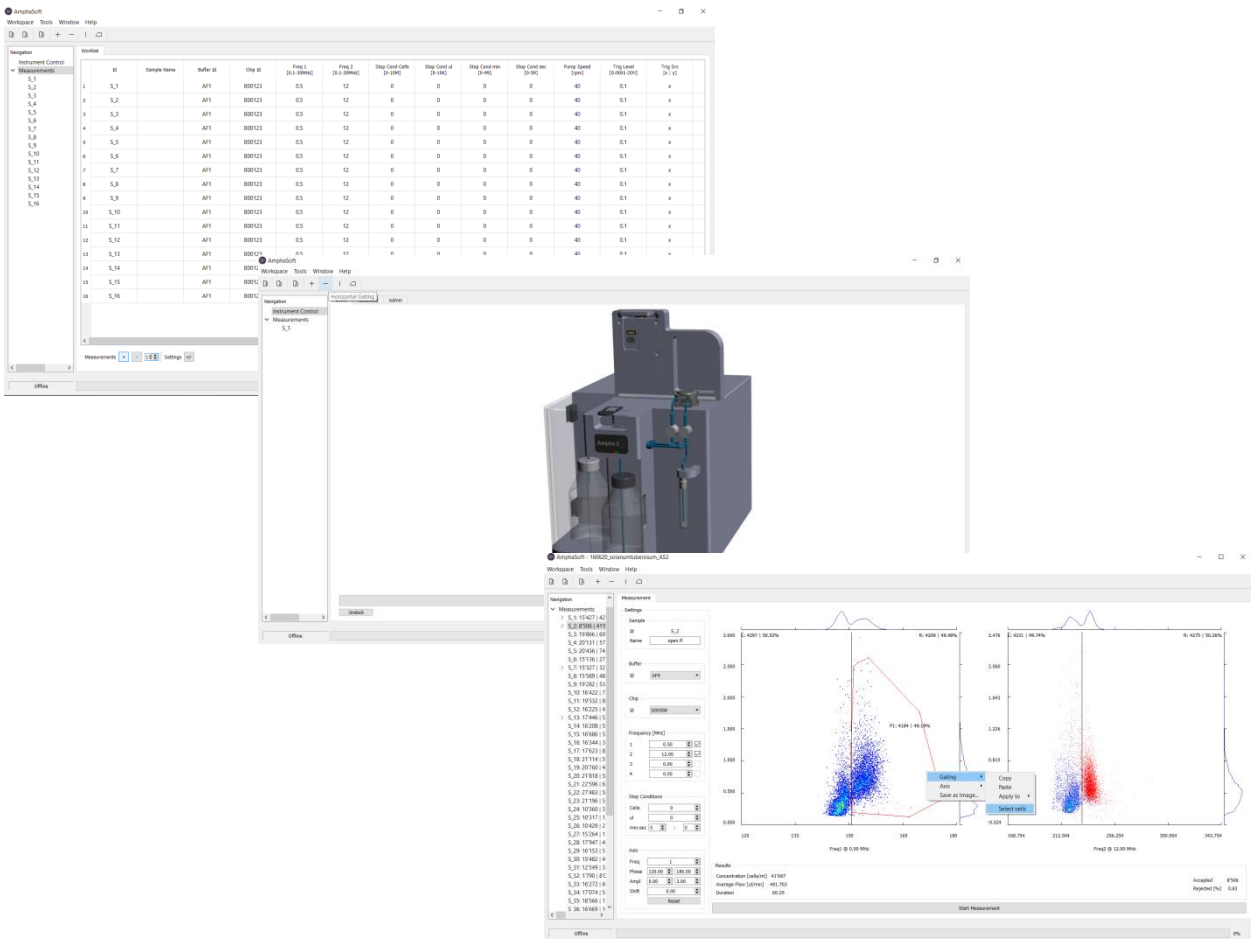


# AMPHASOFT 2.0

# SOFTWARE USER GUIDE

## Release 2.1.6





## Contents

<b>1</b>	<b>Introduction.....</b>	<b>4</b>
<b>2</b>	<b>AmphaSoft 2.0 Software Structure .....</b>	<b>5</b>
2.1	Operation Modes .....	5
2.2	Starting AmphaSoft 2.0.....	5
2.3	The Workspace Concept .....	6
2.4	Static Software Components .....	6
2.4.1	Main Menu .....	6
2.4.2	Navigation Panel.....	7
2.4.3	Status and Progress Bar.....	7
2.5	Dynamic Software Components (tabs) .....	7
2.5.1	The <i>Measurement</i> tab.....	8
2.5.2	The <i>Worklist</i> .....	12
2.5.3	The <i>Basic</i> tab.....	17
2.5.4	The <i>Advanced</i> tab.....	19
2.5.5	The <i>Admin</i> tab .....	23
2.6	Data Saving .....	24
2.7	File Structure .....	24
<b>3</b>	<b>Measuring with AmphaSoft 2.0 .....</b>	<b>26</b>
3.1	Software Structure – Introduction to the Main Components.....	26
3.2	Instrument and Software Setup .....	27
3.3	Experiment Setup .....	28
3.3.1	Single measurement with default settings .....	28
3.3.2	Measurements with customized settings .....	29
3.3.3	Continuing measuring using an existing Workspace .....	31
3.3.4	Counting mode .....	31
<b>4</b>	<b>Creating and Using Templates.....</b>	<b>33</b>
4.1	Creating a New Template .....	33
4.2	Loading a Template .....	33
<b>5</b>	<b>Data Analysis .....</b>	<b>34</b>
5.1	Changing the Number of Plots.....	34
5.2	Adjusting the Scatterplot Characteristics .....	34
5.2.1	Scatterplot Axes (Zooming) .....	34
5.2.2	Size of Data Points .....	35
5.2.3	Density Plot Appearance .....	35
5.3	Gating .....	35
5.4	Hide Cells (Professional license required) .....	37
5.4.1	Hiding .....	38



5.4.2	Unhiding.....	38
5.5	Advanced Gate Statistics (Professional license required) .....	38
5.6	Overlay of plots (Professional license required).....	39
5.7	Exporting Plots.....	39
5.8	Exporting Impedance Data (Professional license required) .....	40
5.9	Reporting .....	40
<b>6</b>	<b>Chip Tests .....</b>	<b>43</b>
6.1	Measurement Chip Test.....	43
6.1.1	Performing a Measurement Chip Test .....	43
6.1.2	Chip Information .....	44
6.2	CalibChip Test .....	45
6.2.1	Performing a CalibChip Test.....	45
6.2.2	Performing a Pulse Test .....	46
<b>7</b>	<b>Transferring Workspaces from AmphaSoft 1.2.8 to AmphaSoft 2.0 .....</b>	<b>48</b>
<b>8</b>	<b>Installation and Updates .....</b>	<b>49</b>
8.1	System Requirements .....	49
8.2	AmphaSoft 2.0 PC Software Installation .....	49
8.3	AmphaSoft 2.0 PC Software Update .....	50
8.4	Instrument Software and Firmware Updates.....	51
<b>9</b>	<b>Licensing .....</b>	<b>54</b>
9.1	Uploading a License .....	54
9.2	Activation of a License .....	55
9.3	Deactivation of a License.....	55
9.4	AmphaFlex.....	56
<b>10</b>	<b>Troubleshooting .....</b>	<b>57</b>
<b>11</b>	<b>Warranty and Technical Support .....</b>	<b>58</b>
11.1	Limited Warranty.....	58
11.2	Extended Warranty .....	58
11.3	Support .....	58



## 1 Introduction

AmphaSoft 2.0 is the software used to operate the Ampha Z32 Impedance Flow Cytometer. It is used for any operation of the instrument, such as setting up measurements, performing measurements, data analysis, reporting and maintenance. AmphaSoft 2.0 runs on a Windows-based laptop PC. The name AmphaSoft 2.0 includes all releases 2.x.y.

The PC software and the corresponding instrument software and firmware are available from the Amphasys website. Software licenses can be obtained from Amphasys.

For more information about the Ampha Z32 Impedance Flow Cytometer, accessories, chips, etc., please refer to the *Ampha Z32 User Guide* or visit our website <https://amphasys.com/ampha-z32-pollen-analyzer/>.

### Licenses

The operation of the Ampha Z32 Impedance Flow Cytometer requires a license. Amphasys provides several types of licenses:

- **Standard**

This single license allows online instrument control (measurements and basic data analysis) using 1 PC.

- **Professional**

The professional licenses come in a bundle of 3 licenses and allow 3 PCs to connect to 1 instrument for online and offline operation with extended data analysis options.

- **Measurement - AmphaFlex**

License is limited to a predefined number of measurements and / or time period, e.g. 1 month or year and can be updated.

More information about the licensing can be found in chapter *9 Licensing*.

### Autosampler

The setup and use of the Ampha Z32 in combination with the Autosampler is described in the *Ampha Z32 User Guide*.



## 2 AmphaSoft 2.0 Software Structure

This section provides detailed information about AmphaSoft 2.0 software structure, features and buttons. If you intend to directly start a measurement, *chapter 3* will guide you through the setup steps.

### 2.1 Operation Modes

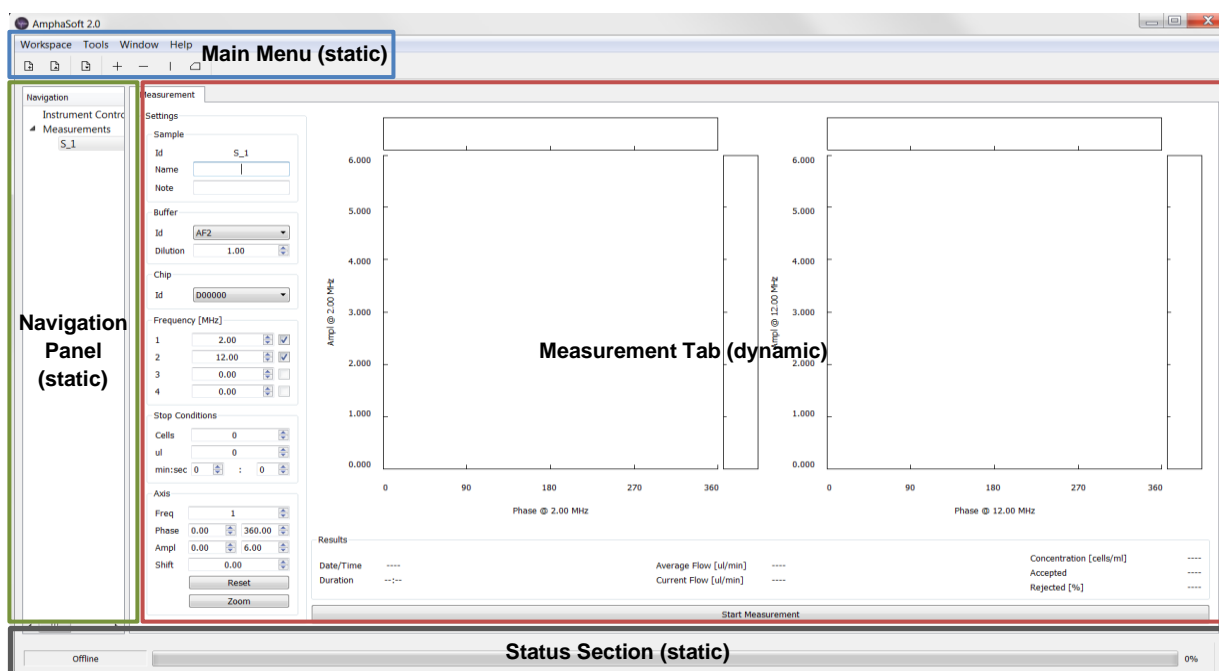
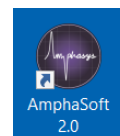
AmphaSoft 2.0 operates in either **Online** or **Offline** mode. *Online* refers to the state when a running instrument is connected to the PC. On the other hand, *offline* operation does not require a connection to the instrument. The use of a standard license only allows online operation, while other license types allow both offline and online operations.

Online	Offline
<ul style="list-style-type: none"><li>▪ <b>Data Acquisition</b></li><li>▪ Planning of Experiments</li><li>▪ Data Analysis</li><li>▪ Reporting</li></ul>	<ul style="list-style-type: none"><li>▪ Planning of Experiments</li><li>▪ Data Analysis</li><li>▪ Reporting</li></ul>

Further information about the Ampha Z32 instrument is supplied in the separate *Ampha Z32 User Guide*.

### 2.2 Starting AmphaSoft 2.0

After installation (see section 8.2 *AmphaSoft 2.0 PC Software Installation*), AmphaSoft 2.0 can be started by double-clicking the AmphaSoft icon (Figure) on your desktop. After a quick loading phase, the main graphical user interface (GUI) is opened (Figure below).



## 2.3 The Workspace Concept

Roughly, the AmphaSoft 2.0 GUI can be divided into static components that are always visible and dynamic components that are only displayed when selected. Static components comprise the *Main Menu*, the *Navigation* panel and the *Status* section. Dynamic components are organized as individual tabs that can be selected. Examples include the *Measurement*, *Basic*, *Admin* and *Advanced* tabs.

All these tabs are used to configure the instrument and to perform operations with a workspace. A workspace contains measurement settings and measurement data. For each experiment, a workspace is created by copying a workspace template and saved in a folder. This workspace can be loaded, modified and saved.

## 2.4 Static Software Components


After starting AmphaSoft 2.0, the user faces the main software GUI, the *Measurement* tab. The *Measurement* tab is surrounded by the static software components, namely *Main Menu*, *Navigation* panel and *Status* section.

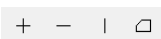
### 2.4.1 Main Menu



The *Main Menu* is located in the top part of the GUI. The menu is divided into 4 sub-menus and 7 icons. The icons are used for workspace management and data analysis.

The 4 sub-menus are:

- **Workspace** Create, load and save workspaces, save workspaces as templates or exit the software. You can also use the 3 icons  (New / Load / Save) to manage workspaces.
- **Tools** In the Tools menu, you can start a Chip Test to check the quality of your chips. A dialogue will open and instruct how to perform the test. Details about the Chip Test are explained in section 6 *Chip Tests*.

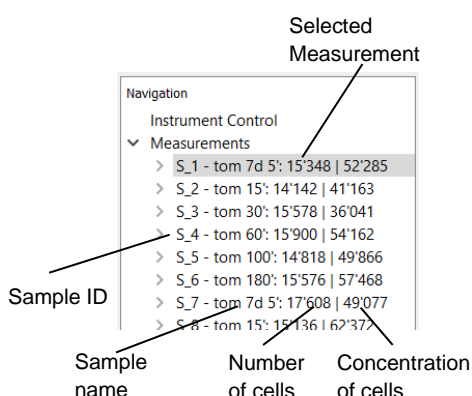
If you select *Gating*, you can apply gatings to your plots for data analysis. You can choose between polygon, cross, horizontal and vertical gates. You can also use the 4 icons  to choose gates. More information about data analysis is provided in section 5 *Data Analysis*.

- **Window** After setting up your preferred window layout (by undocking, adjusting and repositioning tabs), you can save this configuration by clicking *Window > Layout > Save*. When reopening AmphaSoft 2.0, retrieve your preferred layout by selecting *Window > Layout > Load*.
- **Help** Access software or instrument user guides. Check your software release and license type under *Help > About*.

## 2.4.2 Navigation Panel

The *Navigation* panel is used to navigate between individual tabs and measurements. It is divided into 3 parts:

- **Instrument Control** Clicking on *Instrument Control* opens access to the 3 tabs *Basic*, *Advanced* and *Admin*.
- **Measurements** Opens the *Worklist* for experimental setup.
- **S\_1** Each measurement has an ID. S\_1 is the ID of the first measurement. Clicking on S\_1 opens this particular measurement in the *Measurement* tab. In a series of measurements, a list of S\_1, S\_2, S\_3... will be visible. Individual measurements can be selected by clicking on their ID.



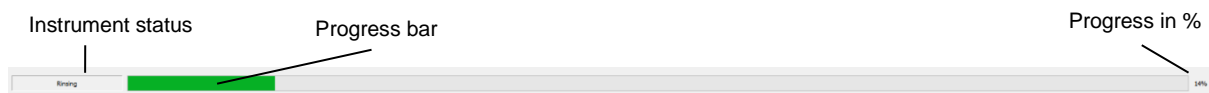
### Measurement List

In a series of measurements, individual measurements can be selected and their results will be displayed in the Measurement tab. The numbers next to the Sample ID and name are the number of measured cells and the concentration of cells of the sample. These numbers will be displayed after stopping the measurement.

Note that you can adjust the size of the *Navigation* panel by moving the separator between *Navigation* panel and the tab on the right.

## 2.4.3 Status and Progress Bar

The status section at the bottom of the GUI consists of a status field and a progress bar. The status field provides information about the instrument status, e.g. “offline”, “rinsing” or “ready”. The progress bar shows the progress of actions, such as the loading process or rinsing cycles.



## 2.5 Dynamic Software Components (tabs)

Dynamic software components are the tabs that can be opened and closed. AmphaSoft 2.0 has the following tabs:

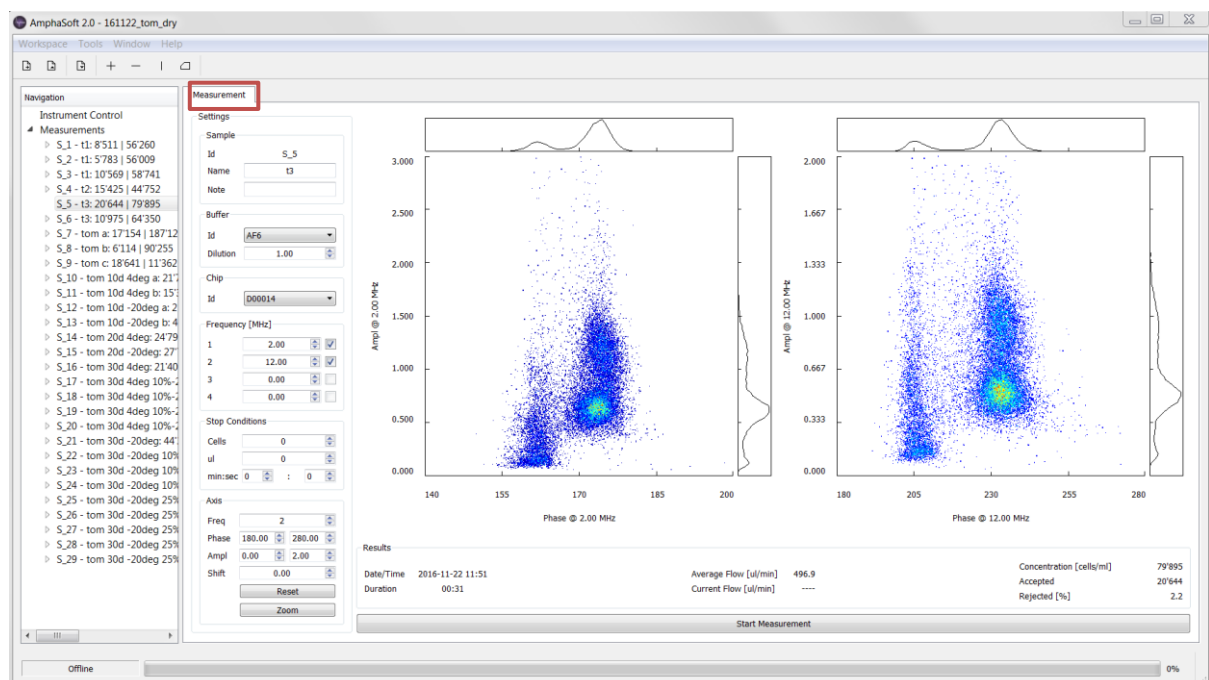
- **Measurement tab** The *Measurement* tab is used to start measurements, view measurement results and perform data analysis.

- **Worklist** In the *Worklist*, measurements can be added and deleted, and measurement settings can be changed. In addition, measurement reports can be generated in both .csv and .html format.
- **Instrument Control – Basic tab** The *Basic* tab of the instrument control is used to start rinsing, cleaning or disinfection cycles. In addition, it shows the sample flow rate in real time.
- **Instrument Control – Advanced tab** The *Advanced* tab contains measurement-specific settings as well as general settings. A large plot can be used to display impedance signals from your sample.
- **Instrument Control – Admin tab** The *Admin* tab is used for instrument software updates, for license activation and to manually disconnect or connect to the instrument. In addition, the tubing set can be selected here.

In the following sections, these 5 tabs and their contents will be explained in depth.

## 2.5.1 The Measurement tab

The *Measurement* tab is used for performing a measurement, result display and data analysis. This tab always displays one single measurement. During a measurement, it provides real-time information about measurement parameters (e.g. average flow rate, number of measured cells...). This tab can also be used to set up a measurement. However, only the worklist provides the full range of configuration options. Start and stop measurements using the bar at the bottom of the tab.



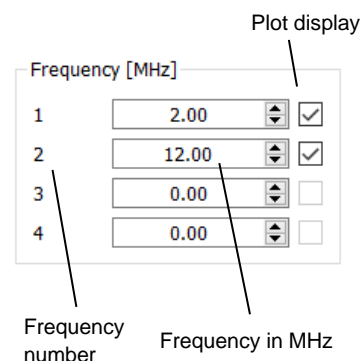
The left part of the Measurement tab is divided into 6 sections:

- **Sample** Displays the sample ID, the sample name and a note field. The sample ID cannot be changed and is consecutive. Each sample should be named appropriately.

- **Buffer** Choose the buffer of your sample from the dropdown-list. If your buffer is not in that list, enter it manually in the *Worklist* by creating a new list element (see section 2.5.2 *The Worklist*). A newly added buffer will be available for all other samples from the dropdown list. In case you work with cells in suspension and you diluted the sample with a measurement buffer, you can enter a dilution factor here to automatically calculate the undiluted cell concentration.
- **Chip** Choose the type of chip you intend to use with the sample. You can use the dropdown menu to just select the chip type (A00000, B00000, C00000...). However, it is recommended to enter the full chip number (e.g. D00123). This can be done in the *Worklist*. After entering a new chip name in the *Worklist*, this name can be chosen from the dropdown for other samples. Changing the chip type leads to the adaptation of default measurement parameters, such as the pump speed.

**Note** The instrument will check if a chip of the selected type (e.g. type D or E) is present in the chip holder. Please make sure that there is no mismatch between the chip selection and the chip you place.

- **Frequency (MHz)** Choose the frequencies with which you want to measure. The Ampha Z32 instrument can measure 1, 2 or 4 frequencies simultaneously. As one frequency typically may not give sufficient information and 4 frequencies increase the noise level of the system, two frequencies are a good choice for most applications. Typically, a low triggering frequency is entered into the first frequency field (e.g. 2 MHz) and a higher frequency (e.g. 12 MHz) in the second field to give additional information. 2 MHz and 12 MHz are the default settings.



### Entering and selecting frequencies

Frequencies can be entered manually in the frequency fields 1 to 4. They must be entered in increasing order. The checkboxes on the right have only graphical implications. Each frequency results in one scatterplot. Selecting a frequency leads to the display of that particular plot in the *Measurement* tab. Deselecting one frequency removes the corresponding plot, but the measurement setting is not changed. Remove frequencies by setting them to 0 (zero) MHz and not by deselecting their plot display.

**Note** Changing the triggering frequency (first frequency) and adding additional frequencies may require the adaptation of other parameters, such as the triggering source and direction, level and gain settings. Please do not play around with these settings and contact our application specialists if you feel insecure. These changes are in the Advanced tab possible.

- **Stop Conditions** Stop conditions are criteria that will stop a measurement when the condition is met. Stop conditions can be defined based on the following measurement parameters:
  - **Cells** Measurement stops as soon as a predefined number of cells or particles has been measured (e.g. 10'000 cells)

- **μl** Measurement stops as soon as a certain sample volume has been measured (e.g. 1000 μl)
- **min:sec** Measurement stops as soon as the given time has passed (e.g. 1 min 30 sec).

**Note** There is no need to set stop conditions, as you can always stop manually. If multiple conditions are set, the first one that is reached will stop the measurement (Boolean OR logic). Typically, one or two stop conditions are set.

Stop Conditions

Cells	10000
ul	1000
min:sec	1 : 30

#### Example: Multiple Stop Conditions

This set of stop conditions will stop a measurement, as soon as 10'000 cells have been measured, 1000 μl sample have passed through the chip or 1 minute and 30 seconds have passed (whichever condition arrives first).

- **Axis** The axis section is used to adjust axes of the plots in the *Measurement* tab. The number of displayed plots can be changed in the *Frequency* section. A plot can be activated for changes by either clicking into it, or by selecting it (1, 2, 3 or 4) in the *Freq.* field. Phase and Amplitude lower and upper axis limits are changed by entering the desired values to the 2 fields next to *Phase* and *Ampl* and confirming with Enter. An entry into the *Shift* field will lead to a horizontal shift of the population. The axes can be reset by clicking on the *Reset* button. Clicking on the *Zoom* button scales the axes automatically. When axis limits are set to default, autozooming is activated during the measurement. In case non-default axis limits are set, the autozooming can be activated by clicking on *Zoom* during the measurement. You can deactivate autozooming in the Instrument Control, Advanced tab.

Plot selection

Axis

Freq	2
Phase	180.00 280.00
Ampl	0.00 2.00
Shift	0.00
Reset	
Zoom	

Lower axis limits Upper axis limits Manual Phase Shift

- **Scatterplots** Each cell that crosses the microfluidic chip is interrogated using one or several measurement frequencies. For each frequency, the cell generates a certain response. Therefore, results are obtained from one single cell for each set frequency. Depending on the number of simultaneously applied frequencies, 1 to 4 scatterplots are displayed. You can change the number of displayed plots by checking and unchecking the boxes in the *Frequency* section. Each measured cell will be represented as a dot in these 2-dimensional plots. The x-axis of each scatterplot corresponds to the impedance phase and is labeled with the corresponding frequency (e.g. *Phase @ 2 MHz*). Similarly, the y-axis corresponds to the impedance amplitude and is also labeled with the corresponding frequency (e.g. *Ampl @ 2 MHz*).



You can change the appearance of the data points in several ways. In brief, clicking into the plot activates the plot for changes. Clicking and dragging moves the field of view, mouse scrolling zooms in or out, *ctrl+scrolling* changes the dot size and *alt+scrolling* changes the density plot appearance. More information about data analysis can be found in section 5 *Data Analysis*.

- **Results** The results section is located below the scatterplots. It consists of 5 measurement parameters that are updated automatically during a measurement.

Results					
Date/Time	2016-11-22 11:51	Average Flow [ul/min]	496.9	Concentration [cells/ml]	79'895
Duration	00:31	Current Flow [ul/min]	----	Accepted	20'644
				Rejected [%]	2.2

- **Date/Time** Information about when the measurement was started.
- **Duration** Time since starting the measurement.
- **Average Flow** Calculation of the average flow (in  $\mu\text{l} / \text{min}$ ) based on the amount of sample that has passed through the flow sensor and the duration of the measurement.
- **Current Flow** Shows the current flow rate during the measurement.

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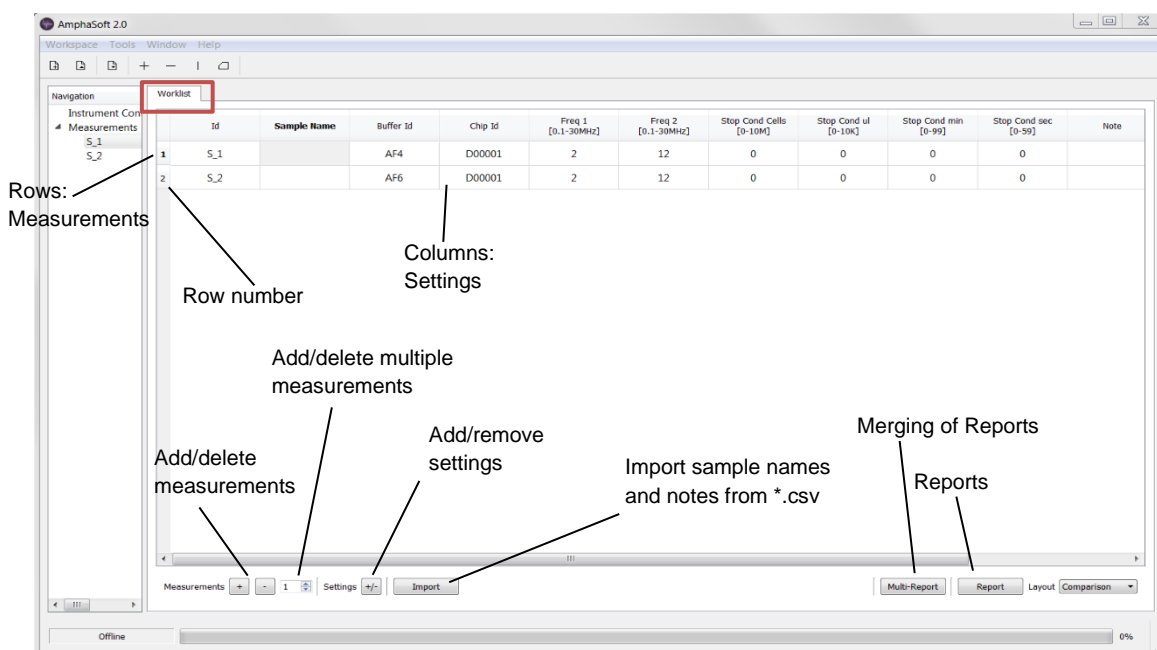
**Note** Air bubbles in the tubing may impede accurate flow rate measurements and therefore affect concentration determinations.

---

- **Concentration** Calculation of the concentration of cells in your sample (in cells / ml) based on the number of accepted cells and the measured volume of liquid that has passed through the flow sensor. After finishing the measurement, the concentration will also be indicated next to the measurement ID, sample name and the number of accepted cells in the *Navigation* panel. The concentration is automatically multiplied with the dilution factor.
  - **Accepted** Number of cells that have been measured since the start of the measurement (after loading). After finishing the measurement, the number of accepted cells will also be indicated next to the measurement ID in the *Navigation* panel.
  - **Rejected** Percentage of signals or partial signals that do not fulfill the triggering criteria. The rejection rate is a good indicator of proper measurement settings (Triggering source, Triggering direction, Level) and may also indicate fluidic problems. In case the rejection rate of a measurement is higher than 15 %, a notification appears after stopping the measurement.
- **Measurement Bar** Below the results section is the measurement bar. This bar is used to start and stop measurements.

## 2.5.2 The Worklist

The worklist is the main tool for planning and setting up experiments. You can access the worklist by clicking on *Measurements* in the *Navigation* panel. The worklist is organized as a table. Each row corresponds to a measurement, and each column corresponds to a particular setting.



### Changing parameters

AmphaSoft 2.0 comes with a set of default parameters that work well for most applications. However, depending on your sample type, adjusted parameters may be beneficial. Ideally you should use a measurement template optimized for your application. A range of application-specific templates are available on the Amphasys website. Alternatively, you have the possibility to edit the parameters in the worklist by double clicking into the field and entering a new value. Please always confirm your entry by pressing *Enter*. The *Buffer ID* and *Chip ID* allow you to select from a dropdown menu or to directly enter your desired description. Your entry will create a new item in the dropdown list that can be selected when setting up other measurements. Use this feature to specify the exact chip number and select it for each measurement you perform with that chip.

### Adding and deleting measurements

Rows can be added or removed by clicking on the (+) or (-) symbols in the bottom section of the worklist. The (+) symbol adds one measurement as a copy in of the last measurement to the list. The (-) symbol removes the last measurement of the list. If you intend to add or remove several measurements, enter the number of measurements in the field next to the add / remove buttons before clicking (+) or (-). You can only add or remove measurements at the end of the worklist.



## Display of custom settings

The worklist allows you to customize the number of settings (columns) that will be displayed. By default, only a limited number of settings are visible. In order to add or remove settings, click on the (+/-) button in the bottom part and select or deselect the desired parameters. Note that only the selected settings will also be mentioned in a report.

## List of parameters

The following section is a list of all parameters that can be set in the worklist with explanations.

- **Measurement Id (Id)** The *Id* is an identification of each sample and should not be changed.

---

**Note** Please do not change sample Ids.

---

- **Well Id (Wells)** This feature is used for autosampler operation only. In the Wells column, a sample can be assigned to a defined well of a 96 well plate.
- **Sample Name** Use the *Sample Name* box to identify your sample by naming it appropriately.
- **Buffer Id** Select or enter the buffer name, in which your sample is suspended. A range of buffers is available from the dropdown-list.
- **Dilution** Enter a dilution factor in case you diluted a cell suspension with measurement buffer. The measured concentration will automatically be multiplied with the dilution factor.
- **Chip ID** Enter the full chip name directly into the field and confirm with *Enter* (e.g. *D00123*, one letter and 5 numbers) or select the chip you intend to use from the dropdown menu in case you already defined it earlier. Changing the chip type leads to the adaptation of the default measurement parameters for each chip.

---

**Note** The instrument will check if a chip of the selected type (e.g. type D or E) is present in the chip holder. Please make sure that there is no mismatch between the chip selection and the chip you place.

---

- **Freq 1** Here you enter the first measurement frequency, which is the default triggering frequency. The impedance signals coming from the triggering frequency will be used to judge if a signal is appropriate (i.e. identified as a cell or particle) or rejected. For most applications, triggering at a low frequency (e.g. 2 MHz, default) is recommended. The frequency range is between 0.1 and 30 MHz.

---

**Note** Changing the triggering frequency may require a change of other parameters, such as the triggering source, triggering direction and level. Please do not play with these parameters if you don't feel confident. You can always ask our application specialists for help. Changes are possible in the Advanced tab.

---

Please do not remove the frequency columns in the worklist.

---

- **Freq 2** Into this field, the second measurement frequency is entered. As the triggering typically occurs at a low frequency, the second measurement frequency is set at higher frequencies (e.g. 12 MHz).



- **Freq 3 and 4** In case you intend to measure with more than 2 frequencies, you can add the third and fourth frequency here (increasing order). Measuring with 3 frequencies is not possible and after entering the third frequency, a fourth identical frequency will automatically be entered. You can however alter this fourth frequency.

---

**Note** Increasing the number of measurement frequencies increases the noise level of the measurement. This decreases the signal to noise properties and further adjustments of the level or gain settings may be beneficial or required.

---

- **Trig Freq** The triggering algorithm uses the impedance data of frequency 1 (usually 2 MHz) by default. If you want another frequency for triggering, change the frequency number here. You may need to reconfigure the triggering algorithm.
- **Stop Cond Cells** You can define a stop condition to stop the measurement automatically, as soon as the criteria are met. The *Stop Cond Cells* is a condition, in which a defined number of accepted cells must be reached. Possible values are between 0 and 10 million.
- **Stop Cond  $\mu$ l** This stop condition refers to the sample volume that passed through the flow sensor. Enter a value between 0 and 10'000  $\mu$ l (microliters) to stop the measurement after this volume has been measured. Use a reasonable value. A microliter stop condition is mandatory for autosampler and counting mode applications.
- **Stop Cond min** This stop condition stops the measurement after a predefined time (in minutes). Any value between 0 and 99 is possible. You can define the time condition more precisely by adding seconds using the *Stop Cond sec* criteria (see below).
- **Stop Cond sec** This stop condition stops the measurement after a predefined time (in seconds) has passed. Any value between 0 and 59 is possible. You can combine this time condition with the *Stop Cond min* criteria (see above).
- **Pump speed** Here you can change the *pump speed* for your measurement (in revolutions per minute, rpm). The pump speed depends on the pump head of your instrument and the chip. Amphasys provides default pump speeds for each chip type (section 6.1.1 *Performing a Measurement Chip Test*). These speeds result in appropriate sampling of your signals and low rejection rates. It is therefore not recommended to change pump speeds.
- **Load** The loading process is used to aspirate the sample and to load it into the chip and flow sensor before the measurement starts. By default *Load* is active (1). In case you want to skip loading and start with the measurement immediately, enter 0 (zero). In the *Counting* mode *Load* is used to drain the lines and to notify you when to place the sample.
- **Counting** The *Counting* mode is a volumetric measurement to determine an accurate sample concentration or obtaining a total cell count of your entire sample. The counting mode is configured by entering 1 (one). It requires a volume stop condition. The volume stop condition must correspond to the volume of sample that you want to measure. The counting mode is only applicable for F, D, E and G chips.

- **Mod** The *Modulation* is the first part of the gain settings. Changing the modulation changes the peak voltage that is applied to the electrodes. Modulation values range from 1 to 5.
- **Ampl** The *Amplification* is the second part of the gain settings. The amplifier is an electronic component that increases the signal strength. Amplification values between 1 and 8 are possible.
- **Dem** The *Demodulation* is the third part of the gain settings. Demodulation is the step, where the information of multiple frequencies is separated. Demodulation values range from 0 to 8. It is recommended to work with low demodulations (0 to 2).

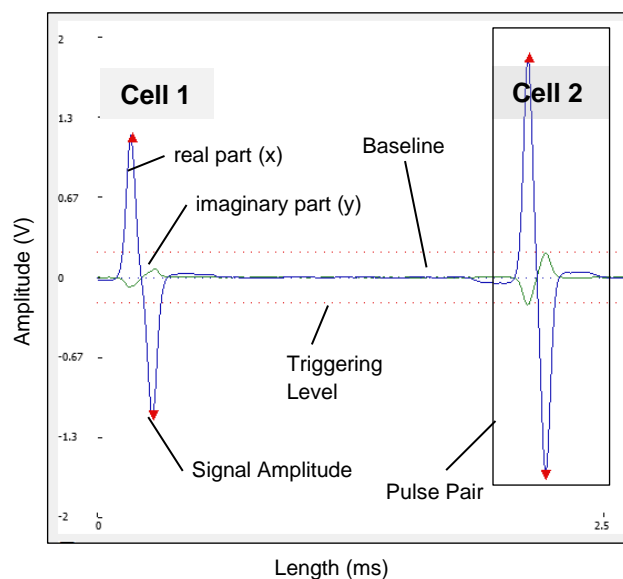
**Note** The gain settings have a strong impact on the signal amplitude and the signal-to-noise ratio. When changing gain settings, please make sure you check your triggering level settings.

- **Trig Level** The *triggering level* is an important setting. It is a criterion that defines which impedance signals are high enough to originate from a cell and which ones are coming from noise or debris. Details are shown in the following figure.

### AmphaSoft 2 Signal Processing

The Ampha Z32 Impedance Flow Cytometer measures impedance signals and then processes these signals using a triggering algorithm. The impedance signals always consist of a real (x, blue) and an imaginary part (y, green).

If there is no cell in the measurement unit, the signal is a baseline at zero amplitude. When cells are crossing the electrodes, impedance signals in the form of opposite double-pulses are measured. The algorithm identifies such pulse pairs and finds their signal amplitude. This data is then further processed and displayed in the scatterplots of the *Measurement* tab.



**Note** The *triggering level* strongly depends on the gain settings. Gain settings modify the size of the signals and the signal-to-noise ratio. If you change the gain, please make sure you also adjust the triggering level accordingly. Setting the triggering level too low will lead to triggering of noise and debris. Setting it too high will lead to the loss of cells. Both scenarios can impede accurate concentration determinations and may bias your results.

- **Trig Src** This parameter is the selection of the *triggering source*. During a measurement, the triggering algorithm will process the signal of the triggering source to find impedance signals from cells. As impedance is a complex quantity, the raw signal of your triggering frequency (Frequency 1) consists of a real and an imaginary part. In the *Advanced* tab, the real part of the triggering frequency is displayed in blue

(x), and the imaginary part in green (y). The component with the higher absolute signal amplitude is typically taken as the triggering source. Depending on your signal properties, select x or y to be the triggering source. For most applications with low triggering frequencies (e.g. 2 MHz), the real part of the signal (x) is the appropriate triggering source.

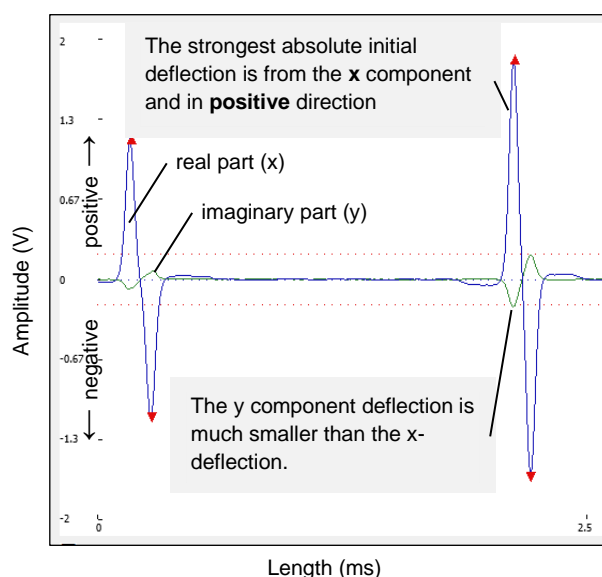
**Note** Please do not adjust the triggering source without knowledge of the signal properties. Contact Amphasys for further questions.

- **Trig Dir** The *triggering direction* is another parameter to configure the triggering algorithm. Depending on the triggering frequency, the selected triggering source (real or imaginary part, x or y) shows signals with initial upward (+) or downward (-) deflections. Use the triggering direction parameter to specify which behavior the algorithm should seek. For most applications with low triggering frequencies (e.g. 2 MHz), positive (+) is the appropriate triggering direction.

**Note** Please do not adjust the triggering direction without knowledge of the signal properties.

### Selecting triggering source and direction

The AmphaSoft 2 triggering algorithm uses either x or y signal components to trigger cellular signals. It is therefore crucial to properly configure the algorithm by selecting the triggering source and triggering direction. The oscilloscope view allows displaying impedance signals. Based on pulse pairs, the triggering source and direction can be determined as shown in the figure to the right. The component with the strongest absolute deflection is used as the triggering source. Depending on whether the initial deflection is in positive or negative direction, the triggering direction is set.



- **Trig Frame** The *triggering frame* is a parameter that influences the triggering algorithm. If a signal is longer than the triggering frame (in milliseconds), it will be rejected. The length of a signal with respect to time is influenced by the particle's velocity. The particle velocity depends on the pump speed, the pump phase (peristalsis) and the position in the chip. **Please do not adjust the triggering frame.**
- **Trig Hyst** The *triggering hysteresis* is a parameter to fine-tune the triggering algorithm. **Please do not adjust the triggering hysteresis.**
- **Resuspension** This feature applies to autosampler operation only. *Resuspension* is a process, in which the autosampler needle releases air bubbles and performs a linear bidirectional movement in order to suspend particles that settled to the bottom of a well. The resuspension time can be adjusted between 0 and 60 seconds.



- **Flush** A *Flush* is a short rinsing cycle of the fluidic system using deionized water from the water bottle. A flush starts immediately after stopping the measurement. If you do not want to flush after the measurement, enter 0 (zero) in the flush field.

---

**Note** Flushing is a cleaning procedure that prevents cross-contamination between samples. Without a flush, there will be particles from the last measurement in the tubing and even after loading the next sample, there may be some residual cells that will be measured with the new measurement. If you decide not to flush, please take such carryover into consideration. For autosampler operation, not flushing does not have adverse effects on carryover.

Note or non-autosampler operation: As the flush starts immediately after the measurement, your sample will be diluted with deionized water unless you quickly remove the sample and place a liquid container below the sample aspiration tube. Dilution with deionized water changes the conductivity of the liquid and the concentration of cells.

---

- **Note** The *Note* field can be used for remarks.
- **Done** The *Done* field is an indicator, if the measurement was already performed. You can't change the entry in the *Done* field.
- **Report** In the *Report* field, you can select if a particular measurement should be included in the measurement report. You can include measurements by entering 1 (default) or exclude by entering 0 (zero).

### Import of sample names and notes

The *Import* function offers the possibility to import sample names and notes into the worklist. The requirements for using the feature are

- Sample names and notes are saved in \*.csv format using a particular separator (comma, @ and semicolon are allowed). Having notes is optional.
- A blank workspace or a template with only one measurement

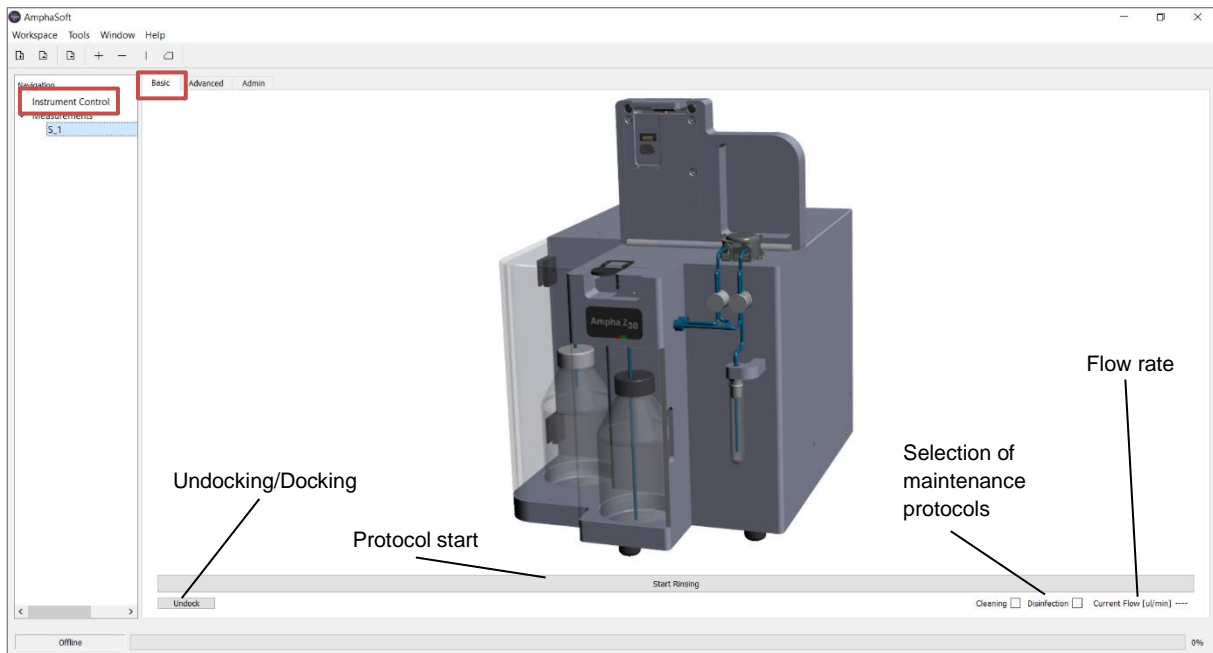
Click on Import and select the \*.csv file containing sample names and notes. The worklist will automatically be extended with the required number of measurements and the corresponding names and notes.

### 2.5.3 The *Basic* tab

The *Basic* tab can be accessed by clicking on *Instrument Control* in the *Navigation* panel and then selecting the *Basic* tab. The *Basic* tab is used to perform the initial rinsing and any other rinsing, cleaning and disinfection cycles. In addition, the *Basic* tab shows real time flow rate information (in µl/min).

## Rinsing

Place a measurement or cleaning chip in the instrument and make sure this chip type is selected in the chip section of the *Measurement* tab. In the *Basic* tab, click *Start Rinsing* and follow the instructions.



## Cleaning

Place a measurement or cleaning chip in the instrument and make sure this chip type is selected in the chip section of the *Measurement* tab. In the *Basic* tab, select *Cleaning*, press *Start Rinsing* and follow the instructions.

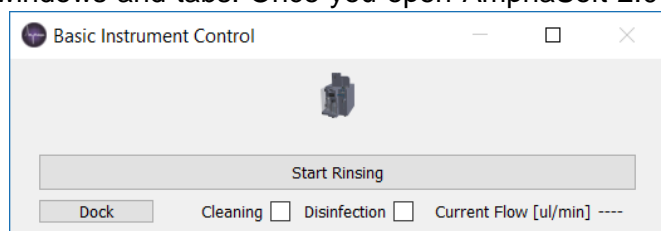
## Disinfection

Place a cleaning chip in the instrument and make sure this chip type is selected in the chip section of the *Measurement* tab. In the *Basic* tab, select *Disinfection*, press *Start Rinsing* and follow the instructions. Only use cleaning chips for the disinfection cycle!

## Undocking

You can undock the basic tab and adjust its size and position. Once you placed the undocked tab at your preferred location, go to the main menu and click *Window > Layout > Save*. This will save the current layout of windows and tabs. Once you open AmphaSoft 2.0 again, retrieve this layout by clicking *Window > Layout > Load*.

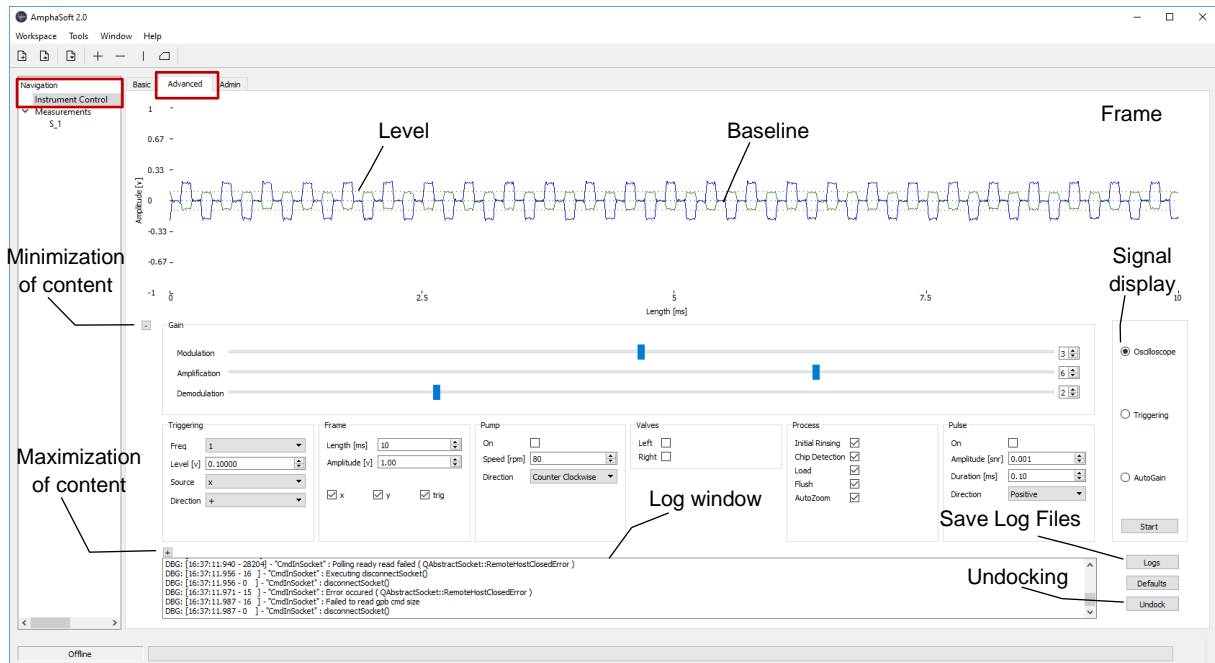
It is helpful to undock the Basic tab and minimize it (picture). In the minimized form, it can always be accessed directly from all other tabs.



If you work with two monitors and decide to use only one (or an external monitor in another position), please save a layout with all windows on your main monitor before changing.

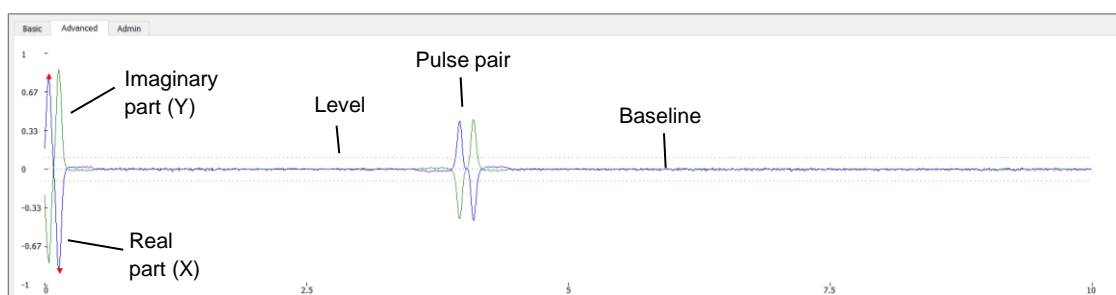
## 2.5.4 The *Advanced* tab

The *Advanced* tab can be accessed by clicking on *Instrument Control* in the *Navigation* panel. It is a tab intended to perform special tasks, such as optimization of triggering settings, viewing signals from cells, manual control of fluidic processes and manual switching of the valves.



### Amplitude-time plot for signal display

The top section of the *Advanced* tab is dominated by a large plot, called the *Frame*. The y-axis corresponds to the signal amplitude (in volts, V) and the x-axis to signal length (time in milliseconds, ms). Note that signals are only displayed when the oscilloscope or triggering view are activated (see next sections). The signals displayed in the plot are the following:



- **Blue line** The blue line corresponds to the real part of the complex impedance signal (the X component). At zero amplitude is the baseline, signal and pulse pairs deviating from that baseline occur as a result of the passage of cells or any particle that impedes current flow.
- **Green line** The green line corresponds to the imaginary part of the impedance (the Y component). A green pulse pair indicates the passage of particles through the chip.



- **Red dotted line** The red dotted horizontal lines correspond to the level and represent a threshold, which a signal must cross in order to be recognized as a true particle signal (and not noise or debris).
- **Red triangles** When the triggering view is activated, examples of triggered pulse pairs are displayed for a short time in the plot. The triggered signal is displayed at time  $t = 0$  and red triangles indicate where the triggering algorithm has determined the signal peak positions. Any other signal in that frame (the plot you see) will not have triangles, even though they may have been triggered.

### Frame section: Plot options

The *Frame* section of the *Advanced* tab is used to change the axes of the frame (the plot in the top section). Using the fields *Length* and *Amplitude*, you can change x and y axes of the frame. Using the check boxes below, you can selectively display real part (x), imaginary part (y) and triggered signals (red triangles).

### Gain section: Change the signal amplitude

The *Gain* section displays the three gain components: Modulation, Amplification and Demodulation. These parameters can be adjusted using the slider or the entry fields. More detailed information about the gain settings is in section 2.5.2 *The Worklist*.

### Triggering section: Modify the triggering algorithm

In the *Triggering* section, you set the parameters based on which the triggering algorithm will determine if a pulse pair is a true event or just noise or debris. More detailed information about the gain settings is in 2.5.2 *The Worklist*.

The screenshot shows the 'Triggering' section with the following settings: Freq is set to 1 (dropdown), Level [v] is set to 0.10000 (numeric field with up/down arrows), Source is set to X (dropdown), and Direction is set to + (dropdown).

### Pump section: Manually activate the pump

The *Pump* section is used to manually activate the pump. The peristaltic pump can be operated at different pump speeds, and in different pumping directions. The default pump speed is counter-clockwise (when viewed from top). You can turn on the pump by clicking the check box. The pump speed can be entered in the *Speed* box (in revolutions per minute, rpm). The pump direction can be selected from the dropdown under *Direction*.

The screenshot shows the 'Pump' section with the following settings: On is an unchecked checkbox, Speed [rpm] is set to 100 (numeric field with up/down arrows), and Direction is set to Counter Clockwise (dropdown).

### Valves section: Manually activate valves

The Ampha Z32 front is equipped with two solenoid pinch valves that alternatively pinch one of the two silicone tubings inserted. Using the checkboxes, you can manually switch the valves. The default position is both unchecked. This pinches the two tubing branches from the water bottle and allows the transport of sample from the sample tubing to the chip. Details of the rinsing protocols are explained in the Ampha Z32 manual.

The screenshot shows the 'Valves' section with two checkboxes: Left (unchecked) and Right (unchecked).



### Process section: Change measurement-associated processes

The *Process* section contains 5 checkboxes. By default, all of them are checked.

- Unchecking the *Initial Rinsing* checkbox makes the initial rinsing not mandatory anymore. Don't use this option with routine analysis.
- Unchecking *Chip Detection* inactivates the chip recognition mechanisms of the Ampha Z32. This holds true for all subsequent measurements.
- Unchecking *Load Sample* prevents sample loading. In that case, the measurement will begin immediately after clicking *Start Measurement*.
- Unchecking *Flush* prevents flushing after that measurement.
- Unchecking *AutoZoom* will prevent automatic setting of axis limits during measurement.

Process	
Initial Rinsing	<input checked="" type="checkbox"/>
Chip Detection	<input checked="" type="checkbox"/>
Load	<input checked="" type="checkbox"/>
Flush	<input checked="" type="checkbox"/>
AutoZoom	<input checked="" type="checkbox"/>

**Note** Changing the settings in the *Process* section is generally not recommended by Amphasys. Appropriate rinsing and flushing cycles are required to maintain a clean fluidic system and to prevent cross-contamination. The chip detection is an important security measure to prevent liquid spills into the instrument when no chip is present. Finally, the loading process is transporting the sample to the chip and therefore an important part of the measurement.

### Pulse section: Creating artificial pulses for advanced testing

The *Pulse* section is used for advanced system testing. In brief, the system creates an electric pulse and this pulse can provide information about the Ampha Z32 electronics. Please do not use the *Pulse* section.

☒ Oscilloscope  
☐ Triggering  
☐ AutoGain

### Oscilloscope view: Real time impedance signals

The oscilloscope is a special feature of the *Advanced* tab. It can be activated on the right hand side of the *Advanced* tab by selecting *Oscilloscope* and then clicking *Start*. When activated, real-time impedance data is shown in the Amplitude-Length plot. As the plot is refreshing after short time intervals, you can get an idea about your signals and noise levels.

**Note** The *oscilloscope view* is very useful for the selection of the *triggering source* and *triggering direction* when measuring with non-default settings. Please make sure that you stop the *oscilloscope view* before leaving the *Advanced* tab or changing to another view.

### Triggering view: Real time triggered signals

Besides the oscilloscope view, where snapshot segments of the data are shown in real-time, the triggering view has a stronger focus on triggered events. When activated, the triggering view selects triggered signals and displays them in the Amplitude-Length plot. As the plot is refreshing after short time intervals, you get an idea about your signal quality (signal-to-noise), signal lengths (transition times) and your level setting.

**Note** The *triggering view* is very useful for the adjustment of level settings and for the identification of electronic contacting problems with the chip (asymmetric signals). Please make sure that you stop the *triggering view* before leaving the *Advanced* tab. Do not activate the triggering view during a measurement.



### Autogain: Finding maximum gain parameters

The *autogain* is a special feature for the optimization of the gain settings. When started, an algorithm will search for the maximum gain settings. You can manually pump a sample through the system and then use *Autogain* to find the highest possible gain settings for that sample type, but the default settings or recommended settings from the pollen list fit mostly for an optimal measurement.

### Log window and Log file retrieval

In the bottom section of the *Advanced* tab is the *Log* window. The log window displays the latest instrument actions and error messages. When setting up measurements with new settings or if

```
+  
WRN: [15:40:49.174 - 4 ] - CrossGate::setLabelOrientation( 10 , 0 )  
WRN: [15:40:49.178 - 4 ] - CrossGate::setLabelOrientation( 11 , 0 )  
WRN: [15:40:49.182 - 4 ] - CrossGate::setLabelOrientation( 12 , 0 )  
DBG: [15:40:49.188 - 6 ] - Recorder: Loading data from "C:/Users/Silvan/  
DBG: [15:40:49.192 - 4 ] - Recorder: No freqs or sample bytes available|
```

you face any problems with measurements, the *Log* window may provide valuable information. In case you have a software or measurement issue to discuss with Amphasys support, a *Log*-file can be saved. Please use the *Logs* button in the bottom right section of the *Advanced* tab.

### Resetting parameters

You can always come back to default settings of the *Advanced* tab by clicking on the *Defaults* button in the lower right corner. Note that this action resets the measurement parameters of the currently selected measurement.

### Undocking the Advanced tab

The *Advanced* tab can be undocked, changed in size and repositioned, similar to the *Basic* tab. For undocking, click on the *Undock* button in the lower right corner. This feature is very useful if more than one screen is available. You can save your preferred configuration by selecting *Window > Layout > Save* in the main menu. After reopening AmphaSoft 2.0, you can always retrieve your layout by clicking *Window > Layout > Load*. If you used an external monitor and intend not to use it anymore, please save the tab configuration for single-screen use first before disconnecting the external screen.

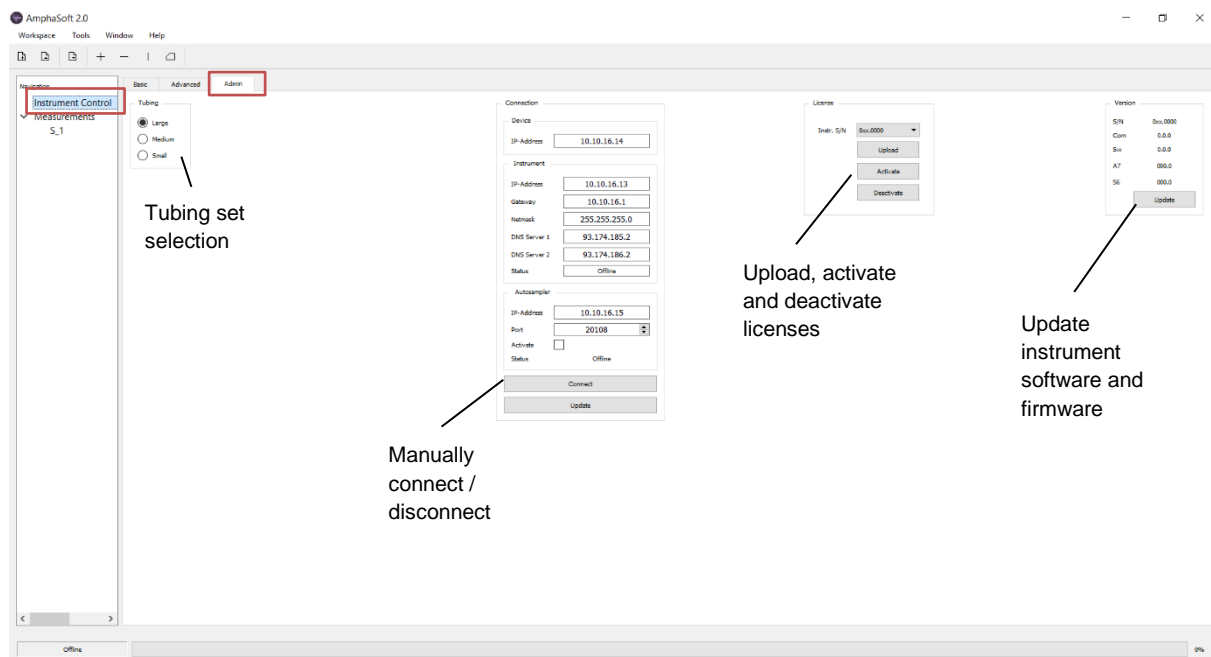
### Minimizing and maximizing Advanced tab contents

The *Advanced* tab contains two buttons to minimize and maximize contents. The [-]-button at the lower left corner of the plot collapses the entire settings and log sections and provides a full view of the plot. You can return to the default view by clicking on the [+] -button.

The [+] -button on the left hand side above the log window leads to an expansion of the log window and the collapse of all the settings. You can return to the default view by clicking on the [-]-button.

## 2.5.5 The *Admin* tab

The *Admin* tab is used for tubing set selection, manual connection and disconnection to the instrument, license activation and instrument software and firmware updates.



### Tubing

Depending on the application, 3 different tubing sets are available. A small tubing set (S) is typically used in combination with A-chips, a medium tubing set (M) with B- and C-chips and a large tubing set (L) with F-, D-, E and G-chips. By default, the large tubing set is selected. If you are using A-, B- or C-chips, you have to adjust the tubing set by selecting it from the list in the *Tubing* section of the *Admin* tab.

### Connection

The *Connection* section contains settings for the communication between your PC and the instrument. The status field tells you if you are working online (i.e. an active connection between PC and instrument) or offline. You can manually connect or disconnect by clicking the *Connect* or *Disconnect* button (only one is available).

In the connection section, the autosampler can be activated. You can find more information in the Ampha Z32 user guide.

**Note** Please do not change any numbers in the *Connections* section of the *Admin* tab. Normally, there is no need to actively connect or disconnect. AmphaSoft 2.0 will automatically try to establish a connection when you perform an operation involving the instrument (i.e. the initial rinsing). AmphaSoft 2.0 will also automatically stop the connection when you exit the software.

## License

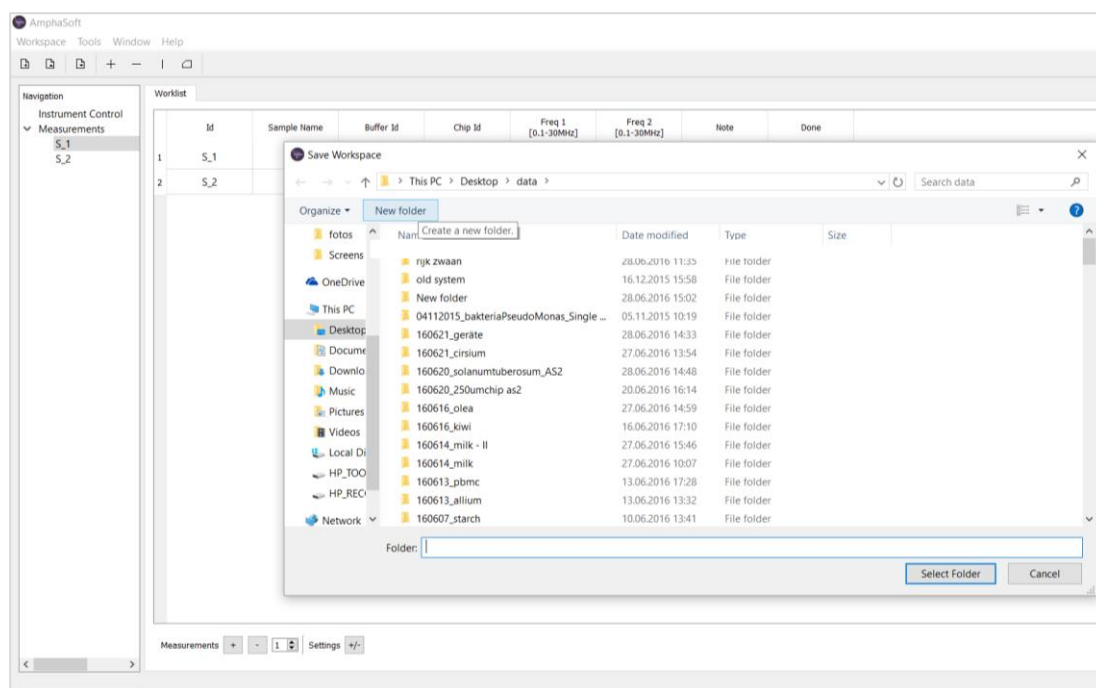
The *License* section of the *Admin* tab gives you the possibility to upload, activate or deactivate licenses. More information about licensing is available in chapter 9 *Licensing*.

## Version

The *Version* section of the *Admin* tab provides information about the configuration of your system and allows you to perform instrument software and firmware updates. Details are provided in chapter 8.4 *Instrument Software and Firmware Update*.

## 2.6 Data Saving

AmphaSoft 2.0 features autosave functionality. It will automatically save the entire workspace after stopping a measurement and after leaving the worklist. However, until the user explicitly saves his work, all files will be stored in a temporary folder. Therefore, only manually saving your work will create a measurement folder. In order to manually save, go to the main menu and select *Workspace > Save*. Alternatively, you can also use the save icon below the main menu. It is good practice to save a workspace before performing the first measurement.



When saving, you will be asked to create a folder. Navigate to your preferred location (use a local but no network drive) and right-click to create a folder (*right-click > New > Folder*). Name the folder and then click *Select Folder* two times. Now you are ready for the measurement.

## 2.7 File Structure

AmphaSoft 2.0 creates a folder for each workspace. The name of the folder can be defined by the user when saving the workspace for the first time (see Section 2.6 *Data Saving*). Inside the folder, 3 files are created for each sample: \*.amph, \*.cels and \*.meas (the “\*” stands for the sample number). These files contain measurement settings and data. In addition, one wsp.amph file is created for the entire workspace.



By default, reports and data exports are also saved in this measurement folder. In case of the *.html* report, an additional subfolder containing the images will be created. When viewing the *.html* report, this subfolder with the images should be saved in the same main folder as the *.html* report.



## 3 Measuring with AmphaSoft 2.0

### 3.1 Software Structure – Introduction to the Main Components

In addition to the detailed information about software structure in *chapter 2*, this section provides a quick overview of the main AmphaSoft 2.0 software components.

#### Measurement tab

After opening AmphaSoft 2.0, the user faces the main menu on top, the *Navigation* panel on the left, and the *Measurement* tab on the right. The *Measurement* tab is used to start measurements, view measurement results and perform data analysis. The *Navigation* panel on the left consists of the *Instrument Control* selection, the *Measurements* selection, and individual measurements, such as *S\_1*.

Clicking on *Instrument Control* opens access to 3 more tabs called *Basic*, *Advanced* and *Admin* (see later). Clicking on *Measurements* opens the *Worklist*, a list of all measurements and their corresponding measurement settings. Clicking on an individual measurement, such as *S\_1*, opens this measurement in the *Measurement* tab.

#### Worklist

The worklist is opened by clicking on *Measurements* in the *Navigation* panel (main window). In the worklist, measurements can be added or deleted, and measurement settings can be changed. In addition, measurement reports can be generated in both .csv and .html format. You can leave the worklist by selecting either *Instrument Control* or an individual measurement from the *Navigation* panel.

#### Instrument Control – *Basic* tab

The *Basic* tab of the instrument control is used to start rinsing, cleaning or disinfection cycles. In addition, it shows the flow rate during any fluidic process in real time. The *Basic* tab can be undocked. You can adjust the size and position of the undocked tab.

#### Instrument Control – *Advanced* tab

The *Advanced* tab contains measurement-specific settings as well as general settings. In addition, the large window can be used to display measurement signals.

#### Instrument Control – *Admin* tab

The *Admin* tab is used for instrument software and firmware updates, for license activation and to manually disconnect or connect to the instrument. In addition, the tubing set can be selected here.

Please only change *Admin* tab settings when you exactly know what you are doing, as these changes can have strong implications on your system. Please do never, unless asked to do so, change any numbers in the *Connection* section of the *Admin* tab.

#### Main Menu

The menu is divided into 4 parts:

- **Workspace** Create, load and save workspaces or exit the software. You can also use the icons below to manage workspaces.



- **Tools** Perform chip tests. Details are explained in chapter 6 *Chip Tests*. Select *Gating* for data analysis. You can also use the 4 icons in the menu bar. Control of the *Autosampler*, details are in the *Ampha Z32 user guide*.
- **Window** After setting up your preferred window layout (by undocking, adjusting and repositioning tabs), you can save this configuration by clicking *Window > Layout > Save*. If you reopen AmphaSoft 2.0, you can click on *Window > Layout > Load* to retrieve your preferred layout.
- **Help** Access software or instrument user guides. Check your software release and license type under *Help > About*.

## 3.2 Instrument and Software Setup

Please also refer to our *Quick Guides* and measurement templates available on the Amphasys website.

- Check the liquid bottles of your Ampha Z32 instrument. The front bottle should contain deionized water. It is used for rinsing steps between individual measurements. The left bottle is a waste container. Please make sure this container is empty.
- If not yet connected, connect your instrument to your laptop using an Ethernet cable (with USB-to-Ethernet adapter).
- Start your laptop, if not yet started.
- Then, switch on your instrument. The switch is located on the rear panel of the instrument.
- Start the software AmphaSoft 2.0 by double clicking the icon on the desktop.
- Perform an initial rinsing step before starting measurements. This step cleans the fluidic system. Make sure that you have an empty liquid container (beaker or 5 ml round-bottom tube) in place under the sample aspiration tube to collect rinsing water.
  - If you are using **F-, D-, E or G-chips** (i.e. you have a L-fluidics set), place a chip in the instrument and close the lid (F, D, E, G or cleaning chip). Select the corresponding Chip ID from the dropdown-menu in the main window. Then, navigate to *Instrument Control* and select the *Basic* tab. Here, click *Start Rinsing* and follow the instructions.
  - In case you are using **A-, B- or C-chips** (i.e. you have a S- or M-fluidics set), click on *Instrument Control* and select the *Admin* tab. Select the appropriate tubing set in the *Tubing* section. Then go back to the measurement panel by clicking on S\_1 in the measurements list on the left. Place one of your chips in the instrument and close the lid (C, B, A or cleaning chip). Select the corresponding Chip ID from the dropdown menu. Then, navigate to *Instrument Control* and select the *Basic* tab. Here, click *Start Rinsing* and follow the instructions.

After the initial rinsing, you can configure and start your experiments.

### 3.3 Experiment Setup


Before starting a new measurement, you need to set your measurement parameters. AmphaSoft 2.0 has default measurement parameters, but depending on your application, optimized settings may be beneficial or required. For details, please refer to the *Ampha Z32 User Guide* or contact Amphasys customer support. Please also refer to our *Quick Guides* and measurement templates available on the Amphasys website.

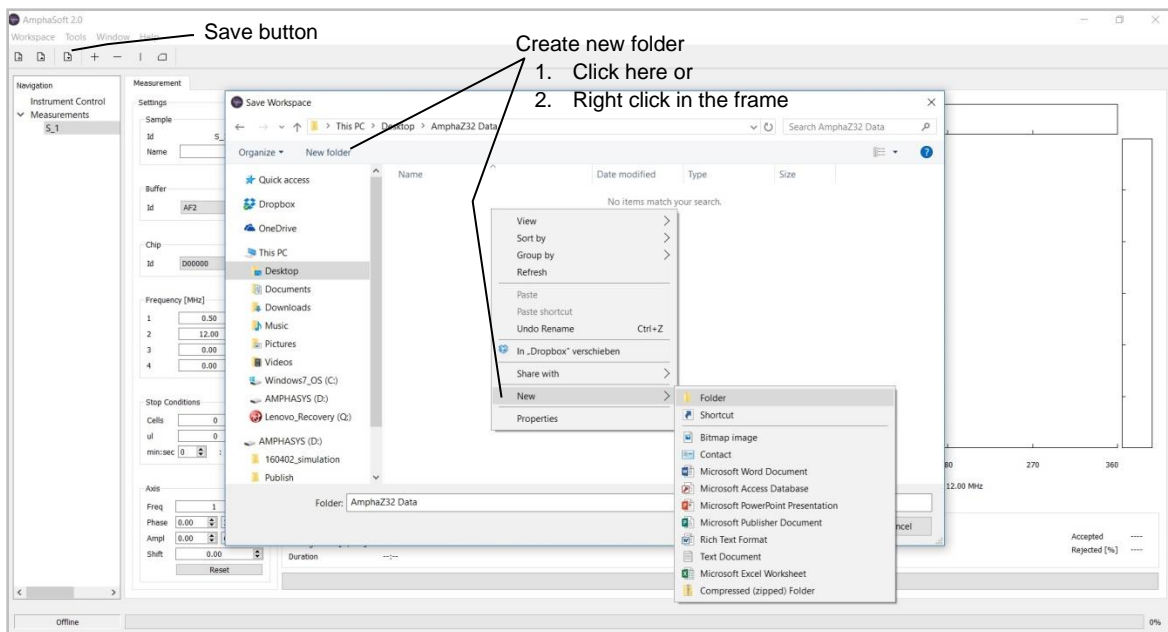
The following sections quickly explain how to set up either a single measurement with default settings, or a series of measurements with adjusted settings. For more detailed information about the individual steps, references to other sections are indicated.

#### 3.3.1 Single measurement with default settings

After the initial rinsing process described in section 3.2, you are ready to measure. It is advisable to always save your workspace prior to the measurement. Otherwise the measurement will be stored only in a temporary folder.

##### Save your workspace

Save your workspace by clicking *Workspace > Save* or using the save-icon . You are asked to create a folder in the explorer view. Navigate to your preferred location and right-click to create a folder (*right-click > New > Folder*). Name the folder and then click *Select Folder* two times. Now you are ready for the measurement.



##### Measurement

- For a single measurement with default settings, select the measurement ID *S\_1* in the measurements list in the *Navigation* panel.
- Prepare the sample, mix it well and place it at the sample holder.



- Press the *Start Measurement* bar at the bottom of the *Measurement* tab.

---

**Note** Do not select any other measurement or tab during a running measurement. This can lead to measurement abort.

---

- You can manually stop the measurement by clicking the *Stop Measurement* bar. Alternatively, you can set stop conditions prior to starting the measurement in the *Stop Conditions* section of the *Measurement* tab.

After clicking *Stop Measurement*, the instrument by default performs a flush. A flush is a short rinsing cycle of the fluidic system using deionized water. After the flush, the instrument is ready for the next measurement. You can either overwrite the current *S\_1* measurement or create a second measurement using the *Worklist*. More information about setting up a measurement series will be provided in the next section (3.3.2 *Measurements with customized settings*). Please also refer to our Quick Guides on the Amphasys website.

For data analysis, you can select the number of plots to display, adjust the axes of your plots, and apply gates to plots etc. In the *Sample* section, you can name your sample by entering a description into the *Name* field. More detailed information about instrument setup and data analysis can be found in sections 2 *AmphaSoft 2.0 Software Structure* and 5 *Data Analysis*. If you do not intend to use the instrument for further measurements, perform a rinsing and an instrument cleaning and/or disinfection. More detailed information about these programs can be found in section 2.5.3 *The Basic tab* and in the *Ampha Z32 User Guide*.

### 3.3.2 Measurements with customized settings

This section explains how to set up a series of measurements with customized settings. First, it is advisable to save your workspace on a regular basis when working. Best practice is to save your workspace before setting up your experiments. Alternatively, you can also save at a later stage, but your workspace will be saved in a temporary folder until you explicitly save.

#### Save your workspace

Save your workspace by clicking *Workspace > Save* or using the save-icon. You are asked to create a folder in the explorer view. Navigate to your preferred location and right-click to create a folder (*right-click > New > Folder*). Name the folder and then click *Select Folder* two times (see images in section 3.3.1)

#### Measurement

A measurement series with default or non-default settings can be created using the *Worklist*. However, Amphasys recommends using preconfigured templates. They can be downloaded on our website.

- Open the worklist by clicking on *Measurements* in the *Navigation* panel
- The *Worklist* is displayed as a table. Each row corresponds to a measurement and each column to a setting. By default, only a few setting parameters are displayed. You can change the number of settings by clicking on the +/- button on the bottom



and then select your desired settings. The settings that are displayed in the *Worklist* will also be displayed in the measurement report.

- Now you can enter your preferred settings. Some fields allow a selection from a dropdown menu. More detailed information about the worklist is available in section 2.5.2 *The Worklist*.
- You can add or delete measurements using the (+) and (–) buttons at the bottom of the window. For the addition or deletion of multiple measurements, you can use the field next to the (+) and (–) buttons and enter the number you want to add or delete before clicking the (+) or (–) button. Measurements are deleted from the bottom up, you cannot delete measurements from the beginning or the middle of the worklist.

Note that you cannot alter the Sample IDs (S\_1, S\_2...). Also note that if you add a measurement, its settings are copies of the last measurement on the list before copying. This feature helps you to quickly create series of measurements with identical settings.

- After finalizing your worklist, return to the *Measurement* tab by clicking on S\_1. You can always go back to the worklist to adjust settings of measurements that have not been done yet.
- Place your sample at the sample holder.
- Click *Start Measurement* to start the first measurement.

---

**Note** Do not select another measurement or tab during a running measurement. This can lead to measurement abort.

---

- If you did not set any stop condition, manually stop the measurement by clicking on *Stop Measurement*. This will start the flush cycle (if selected).

---

**Note** During the flush, rinsing water will be pumped into the waste and out of the sample aspiration tubing. If you want to re-use remaining sample, quickly remove the sample tube from the sample holder after stopping a measurement and place a liquid container under the sample aspiration tube to collect the rinsing water.

---

- After the flush, you can continue measuring by selecting S\_2 in the measurements list, placing the second sample and clicking *Start Measurement*.

Do not forget to save your work after finishing your measurement series and data analysis. You can generate a measurement report including measurement settings and results by clicking on *Report* in the *Worklist*. More information about reporting is available in chapter 5.9 *Reporting*.

If you do not intend to use the instrument for further measurements, perform an instrument rinsing and cleaning and/or disinfection. Detailed information about these programs can be found in section 2.5.3 *The Basic tab* and in the *Ampha Z32 User Guide*.



### 3.3.3 Continuing measuring using an existing Workspace

Instead of creating a new workspace, it is also possible to open an existing workspace and to continue measuring after having added more samples in the worklist. Simply open the existing workspace in the main menu by selecting *Workspace > Load*. Select the workspace folder and click *Select Folder*. After importing, continue as usual with an initial rinsing step and measurements. Amphasys recommends the use of measurement templates with optimized settings. After copying the template, rename the copy and use this copy as your workspace, while leaving the template unchanged.

---

**Note** When loading a workspace, please select the workspace folder and not some internal content of that folder.

---

### 3.3.4 Counting mode

The *Counting* mode is a quantitative measurement mode for accurate concentration and total cell count determination. In the *Counting* mode, the entire sample volume will be measured. The *Counting* mode can be used for F, D, E and G chips. The *Counting* mode can be selected in the worklist settings and should be activated together with the *Load* and *Flush* parameter as well as with *Stop Cond  $\mu$ l*.

Further details and sample preparation recommendations can be found in the *Counting Quick Guide*.

---

**Note** The *Counting* mode is not available for autosampler operation. If you still want to use the feature in autosampler operation, please get in touch with Amphasys support.

---

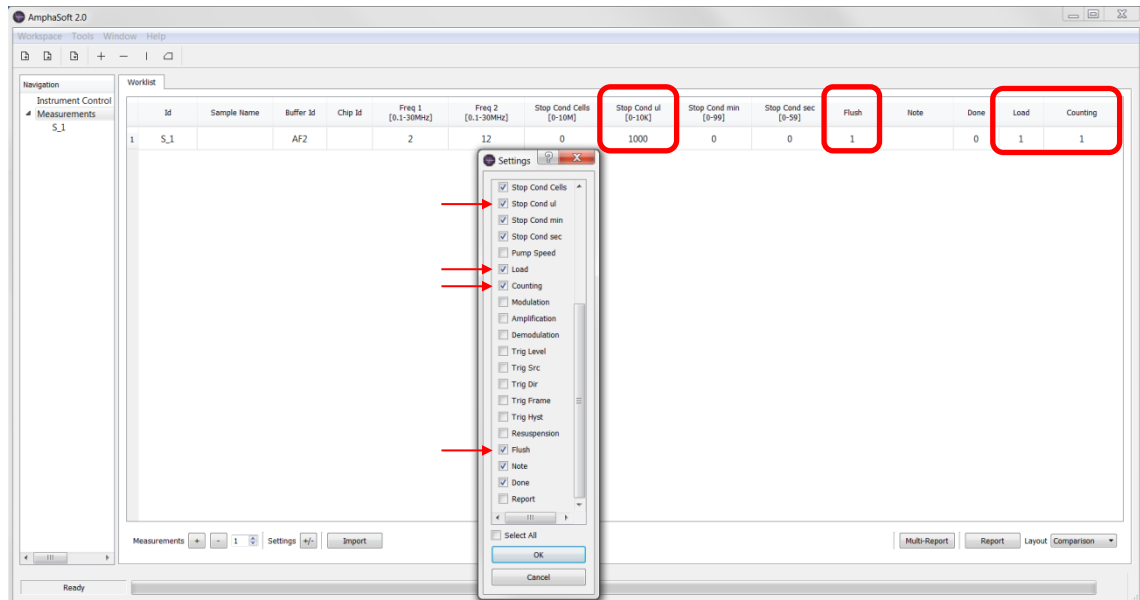
---

**Tip** It is possible to perform *Counting* without *Load* and *Flush* in order to achieve a higher throughput. Please get in touch with Amphasys support to get instructions on how to enter high throughput mode.

---

Amphasys provides species specific Counting templates on the website. However, if you want to configure the Counting mode on your own using your own template, please proceed as described here:

- Open a new workspace and make sure the following columns are displayed in the worklist: *Counting*, *Load*, *Flush* and *Stop Cond  $\mu$ l*.



- *Load* in the worklist should be activated (1), which is needed to drain the lines before the counting sample is loaded. Only deactivate *Load* (0), if you have used *Counting* in the last sample without any flushing or rinsing and the lines are empty.
- Activate/Deactivate *Flush* to clean the lines between the samples. If *Flush* is activated (1), *Load* should be activated too (1).
- Enter your sample volume as a volume stop condition in  $\mu$ l.
- Change to the measurement window by selecting the appropriate sample and start the measurement.
  - If *Load* is activated: There is a notification that no sample tube must be attached to the sample holder. After confirming, the fluidic system is purged with air. Afterwards you are notified to place the sample. Confirm and the measurement starts immediately. It is normal that no cells are detected in the beginning, as the sample must be loaded to the chip first.
  - If *Load* is deactivated: There is a notification to place the sample. Confirm and the measurements starts immediately.
- The measurement lasts at least 30 seconds and stops after no cells have been detected for a period of 10 seconds.
- The concentration indication displays the correct sample concentration based on the number of cells measured and the sample volume configured in the microliter stop condition.



## 4 Creating and Using Templates

Templates are workspaces without any measurement data. Therefore, they mainly consist of a series of experiments with their respective settings and gates. Templates are useful for repetitive measurement series or to set up measurements with identical settings at different geographical sites. As templates do not contain any measurement data, they are very small in size (a few KB) and can be distributed easily.

Amphasys provides a range of species-specific templates on the website. You can download regular templates and templates for *Counting* (see 3.3.4 Counting mode). In case your application is not available, please get in touch with Amphasys support to receive a customized template.

In such customized templates, the name of the species (e.g. tomato) is mentioned in the AmphaSoft header when the template is opened. Therefore, you will always know for which species the template should be used.

### 4.1 Creating a New Template

Finetuning a template requires application know-how and experience. Amphasys supporters are happy to help you with the setup of your application.

The handling of workspaces and templates is also described in the *Quick Guides*.

In case you want to create a template on your own, use a suitable measurement series and export this workspace as a template. This can be achieved in the main menu under *Workspace > Save as Template*. Create a folder using *right-click > New > Folder*, name it and click *Select Folder* two times.

Alternatively, a new workspace can be created and all measurements with their settings can be created in the *Worklist*. Then, this workspace without any data can be saved.

---

**Note** A template is nothing else than an empty workspace. If you open a template and start measuring, you will fill your template with measurement data and lose the empty template. To keep your template, make a copy of it before opening it for measurements or export the workspace after the measurements again as a template.

---

### 4.2 Loading a Template

As templates are empty workspaces, they are also loaded just like normal workspaces. In the main menu, select *Workspace > Load*. Select your template folder and click *Select Folder*. After loading, you can proceed with the initial rinsing and start your measurements.

---

**Tip** You can load workspaces and templates easily by double-clicking on one of the .amph files in a workspace folder. Upon double-clicking, AmphaSoft will launch and display the corresponding measurement.

---



## 5 Data Analysis

AmphaSoft 2.0 features advanced data analysis tools for population gating, quantification and reporting. Most of the data analysis can be performed directly in the *Measurement* tab. Reports can be created in the *Worklist*.

### 5.1 Changing the Number of Plots

The Ampha Z32 Impedance Flow Cytometer can simultaneously apply up to 4 different measurement frequencies. This means that a cell will be interrogated in up to 4 different ways and will give different responses to each frequency. The response to each measurement frequency is displayed in a scatterplot with corresponding histograms in the *Measurement* tab. Therefore, the number of plots that can be displayed is equal to the number of applied frequencies. By default, 2 measurement frequencies are applied. However, also 1 or 4 frequencies can be selected using the *Worklist* or directly in the *Measurement* tab. In the *Measurement* tab, you see which measurement frequencies have been used. The 4 check boxes next to the 4 frequency fields are used to selectively display or remove the plot corresponding to the particular frequency. Therefore, 1 to 4 plots can be displayed simultaneously.

Frequency [MHz]	
1	<input type="text" value="2.00"/> <input checked="" type="checkbox"/>
2	<input type="text" value="12.00"/> <input checked="" type="checkbox"/>
3	<input type="text" value="0.00"/> <input type="checkbox"/>
4	<input type="text" value="0.00"/> <input type="checkbox"/>

**Note** Even though plots may be displayed rectangular, exporting them either manually or in a report will give them a consistent square shape.

### 5.2 Adjusting the Scatterplot Characteristics

The presentation of the measurement data in an ideal way to distinguish populations and to place gates needs scatterplots with optimal data zooming. AmphaSoft 2 allows automated and manual adjustment of the axis.

The scatterplots are autozoomed during the measurement. Autozooming starts automatically when starting a measurement with default axis limits (Phase [0 360] and Amplitude [0 6]) or if the button *Zoom* is activated during measurement. The *Zoom* button is an autozooming feature and displays measurement data with an appropriate zoom factor, making sure that most cells are displayed in a space-filling manner. The plot axis values are regularly updated during measurement until the stop of the measurement.

Each scatterplot can be adjusted also individually. However, most settings can be applied to other plots as well. The following sections explain you how to optimize your plots.

#### 5.2.1 Scatterplot Axes (Zooming)

You can change the phase (x) and amplitude (y) axes of each scatterplot individually either by scrolling with your mouse/touchpad (with the cursor over the plot) or by setting axis limits in the *Axis* section of the *Measurement* tab. The plot to be changed is selected either by clicking into it, or by selecting its plot number in the *Freq* field of the *Axis* section.

In the *Axis* section, you can enter *Amplitude* and *Phase* lower and upper axis limits. Confirm the entry with Enter. The *Shift* field allows you to shift the data points horizontally on the phase axis.

The *Reset* button allows you to reset all zoom settings to default Phases [0 360] and Amplitudes [0 6] of the selected plot.

The *Zoom* button leads to an automatic determination of suitable axes limits. It can be activated during or after a measurement.

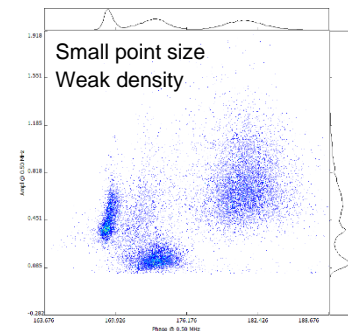
If your axes are applicable to other measurements (e.g. in a series of measurements), you can apply them to others.

- **Apply to all** To apply axis settings to all measurements, right-click into the plot and select *Axis > Apply to > All*. This will copy the axis settings to all plots of the same measurement frequency.
- **Apply to selected** To apply axis settings to only a selected number of measurements, select the measurement with the correct axes. Highlight the desired measurements in the *Navigation* panel using *Ctrl* and then right-click into the plot and select *Axis > Apply to > Selected*.

### 5.2.2 Size of Data Points

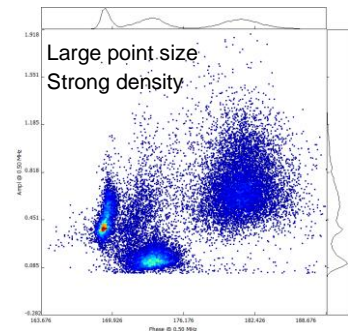
Depending on the number of measured cells, different data point sizes may be appropriate for optimal visualization. Use *Ctrl + scrolling* to change the size of data points.

If your selected point size is applicable to the other measurements, you can apply them to the entire workspace by right-clicking into the plot and selecting *Point Size > Apply to > Workspace*.



### 5.2.3 Density Plot Appearance

Color-coded density information is another feature of AmphaSoft 2.0 that allows attractive data representation and facilitates gating, especially for high cell numbers. A high density of data points in a certain area will result in reddish coloring, while low cell densities are represented by blue coloring. Use *alt + scrolling* to alter the density plot representation.

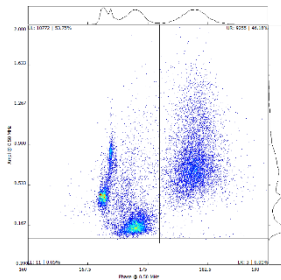


## 5.3 Gating

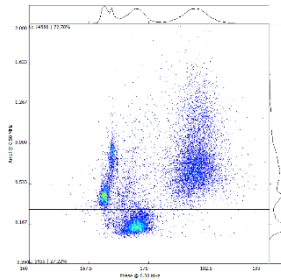
Gating is a way of quantifying populations of data points in scatterplots. AmphaSoft 2.0 offers a range of different gating possibilities, including cross, horizontal, vertical and polygon gatings. A gating can be selected in the *Main Menu* under *Tools > Gating*. Alternatively, the gating icons right below the main menu can be used. Simply select a type of gating and place it in a plot. Note that only one horizontal, vertical or cross-gating can be placed, but several polygon gates are allowed. Line and polygon gates can be combined.

## Gating types

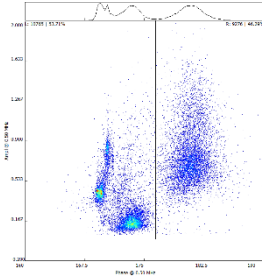
### Cross



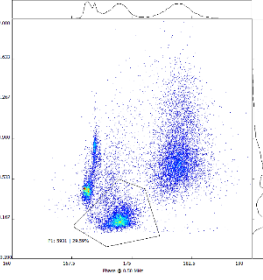
### Horizontal



### Vertical



### Polygon

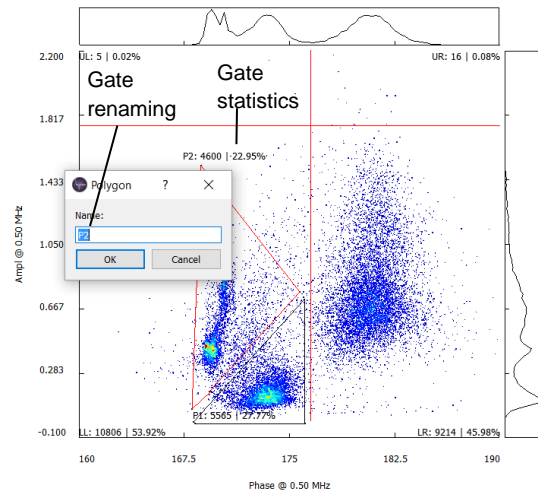


## Gating statistics

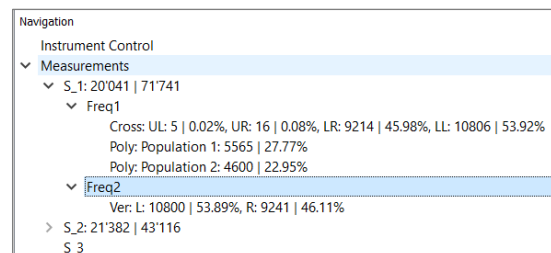
As soon as a gating is placed, gating statistics appear. Gating statistics include the number of data points in the gate and the percentage of this number (of totally accepted cells).

- **Cross-gating** Quadrant statistics quantify the populations in the upper left (UL), upper right (UR), lower left (LL) and lower right (LR) quadrant.
- **Horizontal gating** Statistics for the upper (U) and lower (L) part of the plot
- **Vertical gating** Statistics for the left (L) and right (R) part of the plot
- **Polygon gating** Polygon gate statistics start with the ID of the polygon (e.g. P1, P2...), and then also display the absolute number and percentage of included data points.

Gating statistics can be repositioned by clicking and dragging. Each gating can be renamed by double-clicking on the gating statistics, entering the new name into the popup field and confirming with OK.

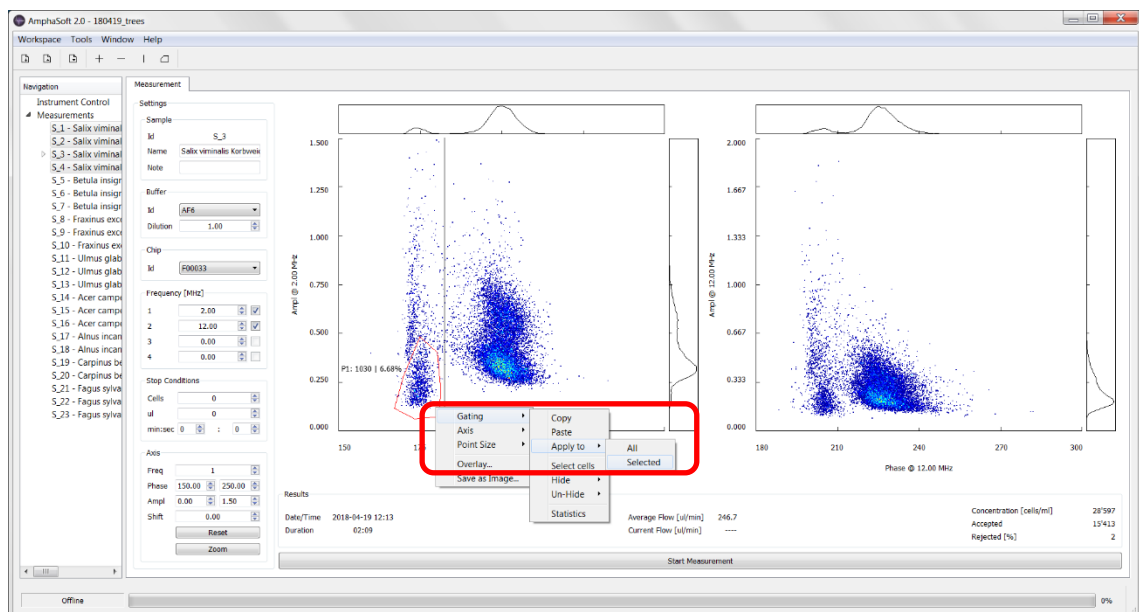


Gating statistics can also be displayed in the *Navigation* panel. Each measurement with a gating receives an arrow (>) in front of its ID. Clicking on the arrow and selecting the frequency using the arrow (v) will reveal all gate statistics of that particular plot.



## Gating operations

- Selecting and adjusting gates** You can reposition gates by selecting them and dragging them to their new location. Cross, horizontal and vertical gates are selected by clicking on them. Polygon gates are selected by clicking into them. You can also adjust the shape of polygon gates by clicking and dragging a corner (a red triangle appears to drag the corner).
- Copy - paste** After placing a gate and selecting it, copying and pasting it into another plot is possible. Therefore, right-click into the plot and select *Gating > Copy*. In the target plot, right-click and select *Gating > Paste*. Alternatively, use keyboard shortcuts *Ctrl + c* for copying, and *Ctrl + v* for pasting.
- Apply to all** If the gate is applicable to the entire workspace, it can be applied to all. Right-click into or on the gate and select *Gating > Apply to > All*.
- Apply to selected** If the gate is applicable to a selected number of measurements, select the measurement with the correct gating. Then, highlight the desired measurements in the *Navigation* panel using *Ctrl* and clicking. Then, right-click into the plot and select *Gating > Apply to > Selected*.

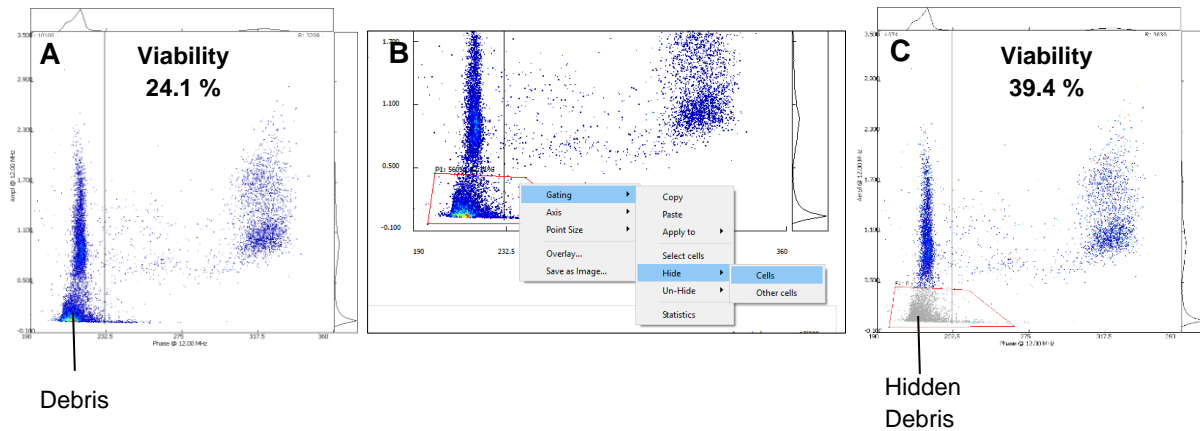


- Select cells** Select cells is a special feature that allows the visualization of a selected population of points in a plot of another frequency. First, define the desired population using a polygon gate. Then, select the polygon gate and use *right-click > Gating > Select Cells*. This action will recolor the selected points red in the other plot. The selection is removed by either clicking *Esc* or selecting another measurement.

## 5.4 Hide Cells (Professional license required)

*Hide Cells* is a useful feature for the exclusion of data from the analysis. It is particularly well suited for the removal of debris. The occurrence of debris complicates data analysis, as the debris particles are false positive cell counts. If a vertical line gate is used for viability analysis, debris particles lead to an underestimation of the cell viability, as they often appear at a similar phase as dead cells (Fig. A).

Using the *Hide* functionality, data points can be excluded from statistics. Each data point has a status, either to be shown or to be hidden. With different operations, this status can be changed.



### 5.4.1 Hiding

To hide cells, create a polygon around the population, select the polygon, *right-click*, select *Gating > Hide > Cells* (Fig. B). The hidden cells will be marked in grey (Fig. C). As hidden cells are not considered in the gate statistics, the statistics will be updated instantly. The polygon gate can be copied to other measurements. Instead of hiding the polygon content, it is also possible to hide everything around the gate by selecting *Hide > Other cells* rather than *Hide > Cells*. Note that this feature is only available for polygon gates.

In case a hiding gate is applied to other measurements, their names and results are greyed out in the measurement panel until the measurement is selected. Selecting a measurement will lead to an update of the accepted number and concentration, accounting for the hidden cells.

### 5.4.2 Unhiding

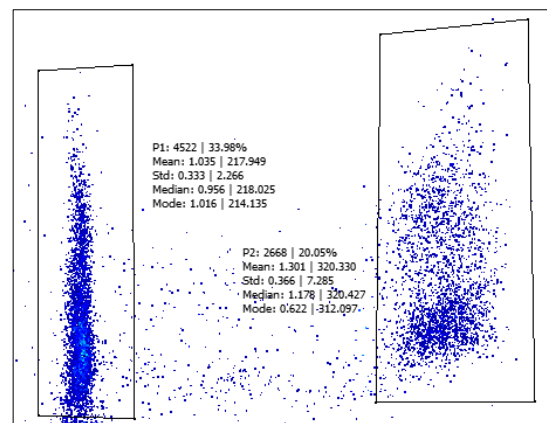
It is always possible to unhide cells by selecting *Un-Hide*. Here is again the option to unhide the gate content or everything around the polygon. In addition, it is possible to unhide all cells in the plot by selecting *Un-Hide > All cells*.

## 5.5 Advanced Gate Statistics (Professional license required)

*Advanced Gate Statistics* is a feature that provides detailed statistical information about the content of a gate, i.e. a subpopulation. *Advanced Gate Statistics* can be displayed by creating a polygon gate around the population of interest, selecting the polygon > *right-click > Gating > Statistics*. Note that this feature is only available for polygon gates.

*Advanced Gate Statistics* include

- **Mean** amplitude and phase



- **Standard deviation** of amplitude and phase
- **Median** amplitude and phase (the central value)
- **Mode** of amplitude and phase (the most frequent value, here the histogram peak position)

The *Advanced Gate Statistics* can be hidden by selecting the polygon > *right-click* > *Gating* > *Statistics*. *Advanced Gate Statistics* will also appear in the reports.

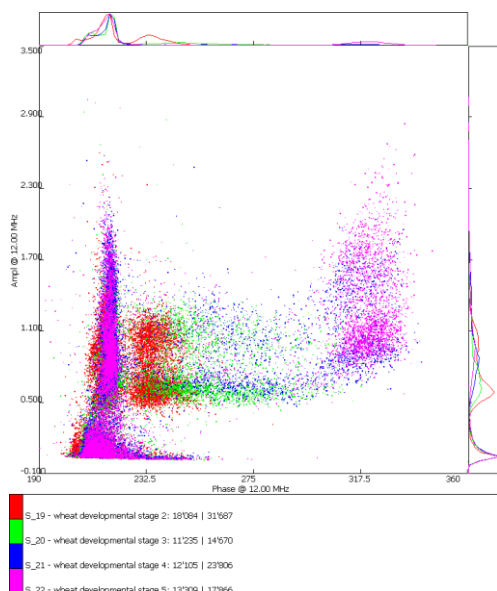
## 5.6 Overlay of plots (Professional license required)

AmphaSoft 2.0 features overlay functionality. This feature is capable of plotting 2 up to 6 datasets into one single plot. It is well suited to visualize a process, e.g.

- Comparing fresh and heat-treated samples
- Visualizing different developmental stages of cells
- Comparing samples containing morphologically different cells (e.g. different ploidy levels of pollen)

To create an overlay, adjust the axes and dot sizes of the plots you intend to overlay to same values. Note that you can only overlay plots with the same frequency index (i.e. the first plot of the first measurement with the first plot of the second measurement).

**Note** Adjusting the axes of all plots to be overlaid is required, as it guarantees the display of data on the same scale.



After adjusting the axes, select all measurements for the overlay (min. 2, max. 6, use ctrl for selecting), *right-click* into the plot > *Overlay...*. A new window will open, displaying the overlay of the selected plots with a new coloring. The legend on the right hand side of the plot indicates which color corresponds to which sample. The histograms are drawn with the same colors. The overlay plot can be saved as \*.png image using the *Save* button at the bottom right. The plot can be closed by closing the overlay window or by clicking *OK*.

## 5.7 Exporting Plots

Besides the reporting feature (section 5.9 *Reporting*) and the overlay feature, AmphaSoft 2.0 allows to export the phase-amplitude plots as \*.png images. Right-click into a plot and select *Save as Image*. Choose your preferred destination and click *Save*. This will create a .png image of the plot, including the histograms and gates.



## 5.8 Exporting Impedance Data (Professional license required)

Impedance data from each measurement can be exported to .csv format for further downstream analysis. The .csv file consists of phase and amplitude pairs for each measured particle. Phases are shown in the first column and amplitudes in the second. The first row corresponds to the first measured particle, the second row to the second particle and so on. The third column contains gate entries. If a particle is inside of a polygon gate, the name of the gate (e.g. "P1") will be shown in the third column. For each measured frequency a .csv file will be generated. The data will be stored in a folder named *export* in the workspace folder. Note that each time an export is made, the content of the export folder will be replaced.

- **Export of data from one measurement**

Right-click on the measurement in the *Navigation* panel and select *Export Cells*. A \*.csv file for each frequency will be generated.

- **Export of data from all measurements**

Right-click on *Measurements* in the *Navigation* panel and select *Export All Cells*. A \*.csv file for each frequency of each measurement will be generated.

## 5.9 Reporting

AmphaSoft 2.0 offers measurement reports in a range of formats. A report in \*.csv format including the measurement settings and results without plots, as well as a report in \*.html format including settings, results and plots can be generated. Only those measurement settings that are displayed in the *worklist* will also appear in the report. Each report format can be created with layouts called *comparison* or *measurement*.

	*.csv report	*.html report
Measurement Settings	•	•
Results	•	•
Gating Statistics	•	•
Plots		•
Merging (Multi-report)	•	

### Report as \*.csv file

In the bottom section of the worklist, click *Report* and then *Save*. This action saves the report with default naming in the measurement folder. Alternatively, you can also select other saving destination and use another filename. After saving, you can open your \*.csv report with appropriate software, e.g. Microsoft Excel.

### Report as \*.html file

In the bottom section of the worklist, click *Report*. Under *Save as type*, select *web (\*.html)* and then *Save*. This action saves the report with default naming in the measurement folder. Alternatively, you can also select another saving destination and use another filename. After saving, you can open the report using your web browser (e.g. Google Chrome or Mozilla Firefox, etc.) for data visualization or printing.

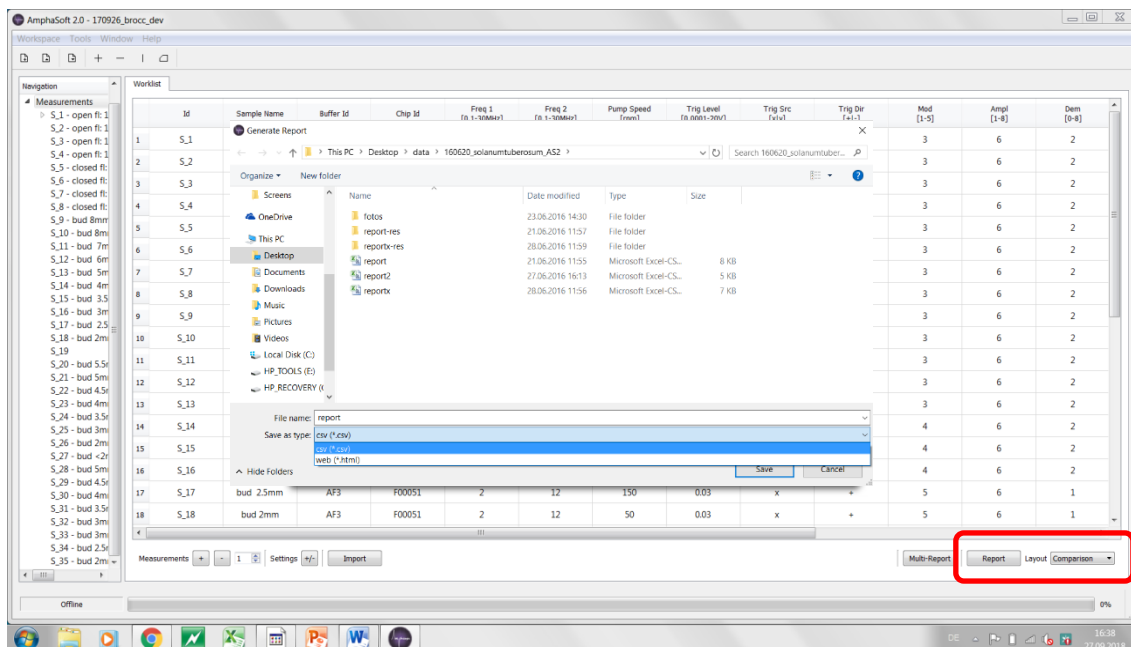
**Note** The \*.html report consists of an \*.html file (default naming: *report.html*) and a folder (default naming: *report-res*). The \*.html file can be opened in a web browser. The folder contains all the images for the report. Therefore, please do not delete the folder. Otherwise your report will open without images. If this happens, you may create the report again.

## Comparison Layout

The comparison layout is intended for measurement series, in which similar cells with similar settings have been measured. This layout is suited for comparing results and plots between measurements. This is the default layout. It can be selected next to the report button.

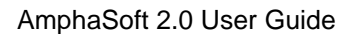
## Measurement Layout

The measurement layout is intended for measurements that don't need comparison. Measurement settings and results are located next to each other for a complete overview of each measurement. This is not the default layout. Select the measurement layout using the menu next to the report button.



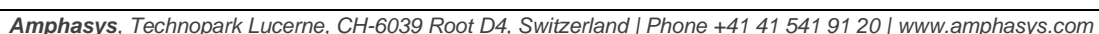
## Row Layout

In the *Row* layout, all settings, measurement results and gate statistics are displayed in a single row for each measurement. The headers of the gate statistics are given in the *Column header assignments* section in the top part of the row report.



## Multi-Report

In a dialog, the desired workspaces can be selected. Make sure all workspaces are saved in the same parent folder. After selecting, AmphaSoft iterates through all measurements of these workspaces. You can specify if all measurements are used or just the ones with a "1" in the report column in the worklist. The merged workspace is saved as .csv in the folder of the first selected workspace as *multi-report.csv*.



## 6 Chip Tests

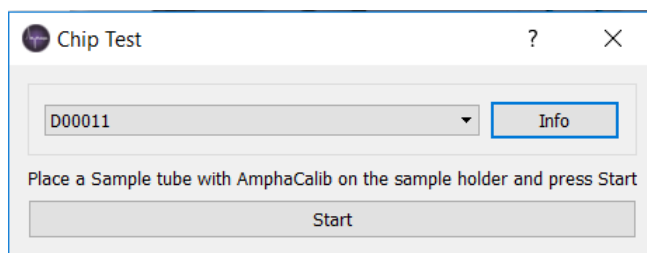
AmphaSoft 2.0 contains a feature to test the electronic system of the Ampha Z32 and the quality of the measurement chips. This feature is called *Chip Test*. The *Chip Test* dialogue can be launched from the *Menu* under *Tools*. Here you can also get detailed information about the chip usage (6.1.2 *Chip Information*.).

### 6.1 Measurement Chip Test

The chip test for measurement chips measures the impedance of a measurement chip filled with a conductive liquid. The liquid for the chip test is AmphaCalib (available from Amphasys). The obtained impedance values are quality criteria and can be used to judge if a chip is suitable for measurement or should be disposed.

#### 6.1.1 Performing a Measurement Chip Test

- Place the chip in the instrument and close the lid.
- Enter the Chip-ID (Chip number) to a measurement in the worklist, when not yet entered
- In the AmphaSoft 2.0 *Main Menu*, select *Tools > Chip Test*.
- Select the Chip-ID from the dropdown menu



- Prepare a 3 ml sample of AmphaCalib and place it at the sample holder.
- Press *Start*.
- After finishing, a pop-up window will display the result of the test.
- The message “Chip Ok” indicates that the chip impedance is within specification limits.
- After a successful chip test perform an instrument rinsing.
- Any other popup than “Chip Ok” indicates a problem with the chip. In case of such a notification, repeat the chip test. If the message is recurrent, try the following steps and repeat again.
  - Make sure that the instrument is pumping liquid (AmphaCalib) through the fluidic system and that there are no air bubbles.
  - Check if the chip is clean, rinse it in the cleaning station with water and if necessary, incubate your chip in AmphaClean solution for 5 to 10 minutes to remove any dirt or crystallization products from the electrode surfaces. Afterwards, thoroughly rinse the chip with deionized water, followed by gentle drying with a lint free cloth or air.

- Clean the instrument electronic pins carefully with a tissue and ethanol.
- If these measures are not successful, please do not use the chip anymore, as its sensitivity decreased or it may not withstand the fluidic pressures of the instrument anymore. Use another chip or contact your local sales representative.
- Note: Impedance values of chip tests are always displayed in the *Log* window of the *Advanced* tab. A table with specification ranges is shown below.

### Chip Impedances in the Log window

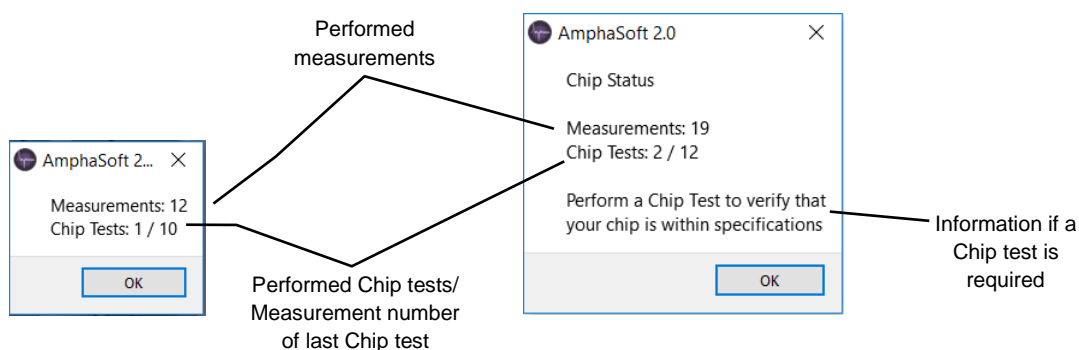
The chip impedance is written as the second last entry. As an example, the D-chip tested here has an impedance of 26.7897 kOhm using AmphaCalib and is therefore already out of the specification range.

```
DBG: [16:06:00.914 - 47713] - Chip Test started
DBG: [16:06:31.348 - 30434] - AutoGain done: Modulation: 5 | Amplification: 1 | De
DBG: [16:06:31.358 - 10 ] - "AutoGain" 100 % done with success
DBG: [16:06:34.366 - 3008 ] - Chip Test done I1: 26.7897 | I2: 0 | I3: 0 | I4: 0 | re
DBG: [16:06:34.383 - 17 ] - "ChipTest" 100 % done with success
```

Impedance of AmphaCalib at 0.5 MHz			
Chip	optimal	acceptable	out of specification
A (15 µm)	80-91 kOhm	91-120 kOhm	> 120 kOhm
B (30 µm)	30-47 kOhm	47-70 kOhm	> 70 kOhm
C (50 µm)	20-38 kOhm	38-50 kOhm	> 50 kOhm
F (80 µm)	18-33 kOhm	33-40 kOhm	> 40 kOhm
D (120 µm)	12-18 kOhm	18-26 kOhm	> 26 kOhm
E (250 µm)	5-12 kOhm	12-18 kOhm	> 18 kOhm
G (400 µm)	3-10 kOhm	10-16 kOhm	> 16 kOhm

### 6.1.2 Chip Information

In the *Chip Test* dialogue, detailed information about the chip usage and quality control is available. In the Chip Test window, select the appropriate Chip ID and press *Info*. You will get a pop up window with following information:



## 6.2 CalibChip Test

In order to test the electronic system of the Ampha Z32, a chip test with calibration chips (CalibChips) can be performed. CalibChips are microchips containing resistors with known impedances. They do not contain any liquid channels. CalibChip tests provide information about your system and the contacting between the Ampha Z32 pins and the chips.

There are two types of CalibChips available:

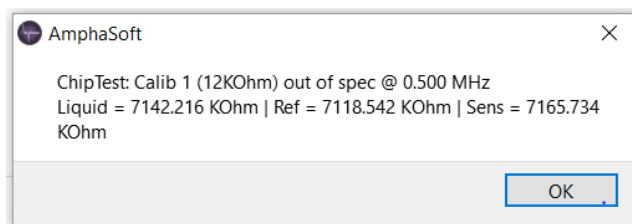
- Calibration chip 1 (12 kOhm)
- Calibration chip 2 (30 kOhm)

### 6.2.1 Performing a CalibChip Test

- Place a CalibChip (12 kOhm or 30 kOhm) in your instrument and close the lid.
- In the AmphaSoft 2.0 *Main Menu*, select *Tools > Chip Test*
- Select the corresponding chip ID from the dropdown list (Calib 1 or Calib 2).
- Press Start
- A popup window will confirm the completion of the chip test and display the chip test result.
- The “Chip Ok” message appears if the chip impedance is within specifications.
- In case of CalibChip test failure, you will be notified with a popup window

**Calibration  
Chip 1**

**Calibration  
Chip 2**



- In case of a failure, repeat the chip test and for recurrent failure contact Amphasys.
- Note: Impedance values of chip tests are always displayed in the *Log* window of the *Advanced* tab after the entry *Chip Test done: |1:*.

```
DBG: [10:31:14.196 - 33131] - Chip Test started
DBG: [10:31:50.268 - 36072] - AutoGain done: Modulation: 3 | Amplification: 1 | Demodulation: 2
DBG: [10:31:50.274 - 6 ] - "AutoGain" 100 % done with success
DBG: [10:31:53.266 - 2992 ] - Chip Test done: |1: 12.2164 | I2: 0 | I3: 0 | I4: 0 | result: 100 %
DBG: [10:31:53.281 - 15 ] - "ChipTest" 100 % done with success
```

- The CalibChip impedances should be in the ranges given in the table below

Impedance Reference Values at 0.5 MHz		
Chip	Optimal (kOhm)	Electronic Problem
Calib 1 (12 kOhm)	11.5 - 12.5	Out of range
Calib 2 (30 kOhm)	29 - 31	

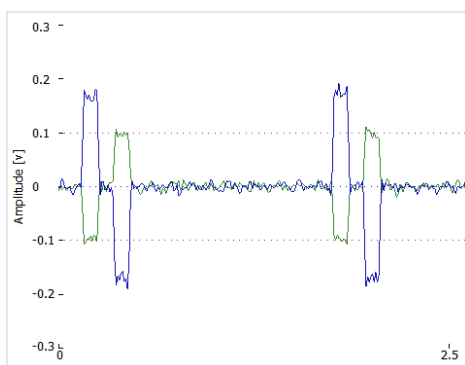
- In case of impedance values out of the specification range, please contact Amphasys.

## 6.2.2 Performing a Pulse Test

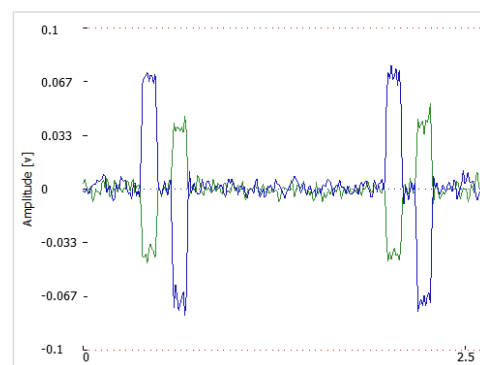
The pulse test is an advanced electronic test.

- Place a CalibChip (12 kOhm or 30 kOhm) in your instrument and close the lid.
- In the AmphaSoft 2.0 *Measurement* tab, select the corresponding chip ID from the dropdown list in the *Chip* section (Calib 1 or Calib 2). Make sure the frequencies are set to 0.5 MHz (Frequency 1) and 12 MHz (Frequency 2).
- In the *Advanced* tab, make sure the gain and pulse settings are set to default (Modulation: 3; Amplification: 6; Demodulation: 2, Pulse Amplitude: 0.001, Pulse Duration 0.1, Direction: positive).
- Turn on the pulse by checking the *On* box in the *Pulse* section
- Turn on the oscilloscope view by selecting *Oscilloscope* and clicking *Start*.
- Adjust the frame Amplitude to 0.3 for the Calib 1 chip and to 0.1 for the Calib 2 chip.
- Compare the pulse shapes and amplitudes using the following screenshots and table.

12 kOhm CalibChip 1



30 kOhm CalibChip 2



**Typical Pulse Amplitudes**

Chip Type	Real part (blue, x)	Imaginary part (green, y)
Calib 1 (12 kOhm)	0.12 – 0.25	0.05 – 0.15
Calib 2 (30 kOhm)	0.05 – 0.09	0.03 – 0.05

- After finishing, stop the oscilloscope view by clicking *Stop*.



## 7 Transferring Workspaces from AmphaSoft 1.2.8 to AmphaSoft 2.0

AmphaSoft 2.0 allows the import of workspaces from AmphaSoft 1.2.8 after a workspace conversion step. For that purpose, AmphaSoft 1.2.8 must be installed on your computer and you need to have a license dongle.

- Open AmphaSoft 1.2.8 in offline mode and load the desired workspace in the main menu under *Workspace > Load*. Select the folder, click *OK* and hit *Enter*.
- Make sure that there are no empty measurements in that workspace. In case of empty measurements, open the worklist and delete the empty measurements. Leave the worklist and save the workspace.
- Then, export the workspace by selecting *Tools > Convert ws for AmphaSoft 2.0*. You will be notified when the conversion has finished.
- A new folder will be created in the same location as the old AmphaSoft workspace. The naming of the new folder has the extension –*AmphaSoft 2.0*.
- After exporting your workspaces, close AmphaSoft 1.2.8.
- Launch AmphaSoft 2.0 and import the workspace using *Workspace > Load*. Select the folder (with extension –AmphaSoft 2.0) and click *Select Folder*.

---

**Note** Data exported from AmphaSoft 1.2.8 will be displayed similarly in AmphaSoft 2.0. However, when acquiring new data, please be aware that AmphaSoft 2.0 introduces a 180 ° phase shift to all data to prevent cloud positioning at the border regions of the phase axis (0° and 360°). Please take this phase shift into account when comparing results from both systems, or when using templates for both systems.

---

## 8 Installation and Updates

AmphaSoft 2.0 consists of a PC software (GUI) and an instrument software and firmware. With new AmphaSoft 2.0 releases, the instrument software and firmware are typically also updated. The installation and update processes are described below.

### 8.1 System Requirements

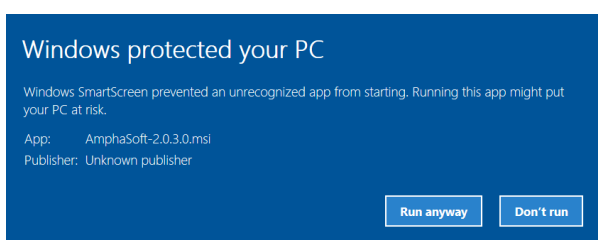
The system requirements for running AmphaSoft 2.0 on a windows-based laptop PC are the following:

- i5 64-bit dual core processor or higher (recommended: i7)
- 8 GB RAM
- At least 128 GB SSD (recommended: 256 GB SSD)
- Full HD screen (screen resolution 1920x1080, recommended: 15' screen)
- Windows 7 or 10 Pro
- Computer name must be in ASCII characters, e.g. AMPHASYS-PC
- OpenGL 3.3 graphics card or higher
- At least 2 free USB 2.0 connectors
- Internet access (WLAN or Ethernet)
- USB-Mouse

### 8.2 AmphaSoft 2.0 PC Software Installation

The following section will guide you through the AmphaSoft software (latest release) installation. It is intended for laptops which do not have any previous version of AmphaSoft installed. You should have administrator rights for your computer in order to install or update AmphaSoft 2.0.

- Download the latest AmphaSoft 2.0 release (\*.msi file) from the download section of the Amphasys website (<https://amphasys.com/downloads/>). In the download section, you can also subscribe for automatic notifications of software updates.
- Start the installation by double-clicking the downloaded installation file (ending with \*.msi) and confirm any administrator or security rights.
- In case the software is not recognized by windows, confirm to execute it. (Click on *More Info* and *Run anyway*).





- After finishing the installation, the AmphaSoft 2.0 shortcut icon is created on your desktop.
- Open AmphaSoft 2.0 by double-clicking the AmphaSoft 2.0 icon and check the AmphaSoft 2.0 software release under *Help > About*.
- Connect your instrument to your PC via an Ethernet cable (and USB adapter).
- Then, switch on your instrument.

**Note** The instrument requires a few seconds to start up. Please do not perform any AmphaSoft software operations right after switching on the instrument.

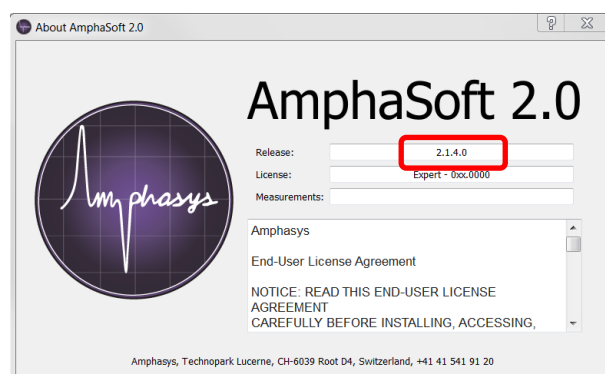
- Press the *Connect* button in the *Admin* tab. Typically, an instrument software and/or firmware update is required after the PC software update. You will be notified about that right after connecting to the instrument. The instrument software/firmware update process is described in section 8.4 *Instrument Software and Firmware Update*.
- After updating instrument software and/or firmware, start the software AmphaSoft 2.0 by double clicking the icon on the desktop.
- In case no license was found, continue in section 9.2 *Activation of a License*.

### 8.3 AmphaSoft 2.0 PC Software Update

AmphaSoft 2.0 updates will be available from the download section of the Amphasys website (<https://amphasys.com/downloads/>). Please check your current software release name in the AmphaSoft 2.0 main menu under *Help > About*.

You should have administrator rights for your computer for updating AmphaSoft 2.0.

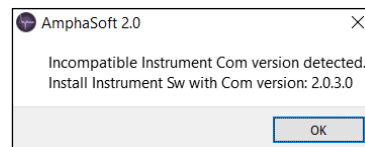
- Download the latest AmphaSoft release (.msi file) from the download section of the Amphasys website (<https://amphasys.com/downloads/>). In the download section, you can also subscribe for automatic notifications of software updates.
- After downloading, start the update by double-clicking the .msi file and confirm any administrator or security rights. The installation usually takes only a few seconds and the old AmphaSoft icon will be replaced.
- Open AmphaSoft 2.0 and check the AmphaSoft 2.0 software release under *Help > About* (e.g. release 2.1.4.0 in the example below). Close the window after checking.



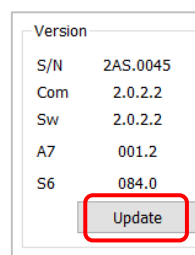
- Continue with the instrument software and firmware updates in the next chapter.

## 8.4 Instrument Software and Firmware Updates

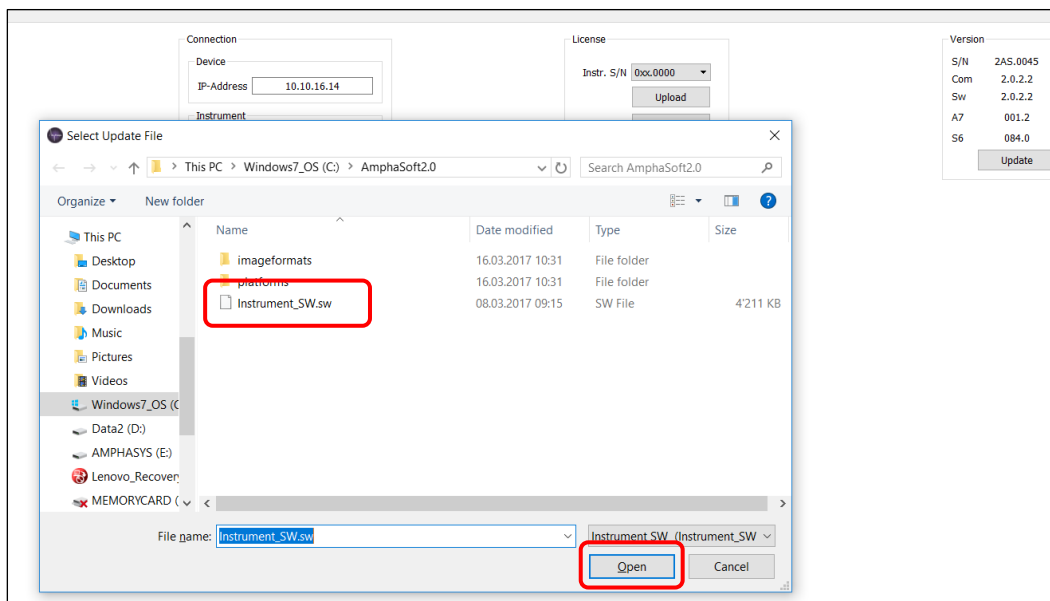
- Typically, an instrument software and/or firmware update is required after the PC software update. Open the *Admin* tab and click the *Connect* button in the *Connection* section. You will be notified in case you need to do an instrument software or firmware update right after connecting to the instrument (see picture below).



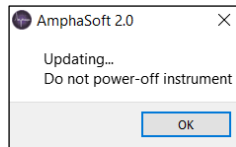
- Confirm the notification by clicking *OK*
- On the **right hand side** of the *Admin* tab is the *Version* section (see image below).



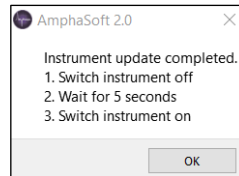
- Click on *Update*
- In the new window, select the file ***Instrument\_SW.sw*** (see image below)
- Click *Open* (see image below).



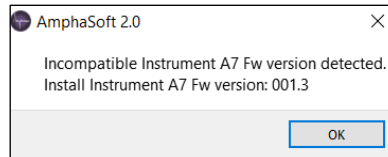
- There will be a reminder not to switch-off the instrument during the process (see image below).



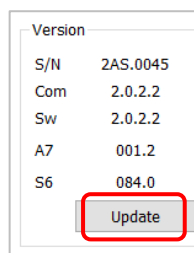
- The update process will take a few seconds and you will be notified about the completion with a popup window (see image below).



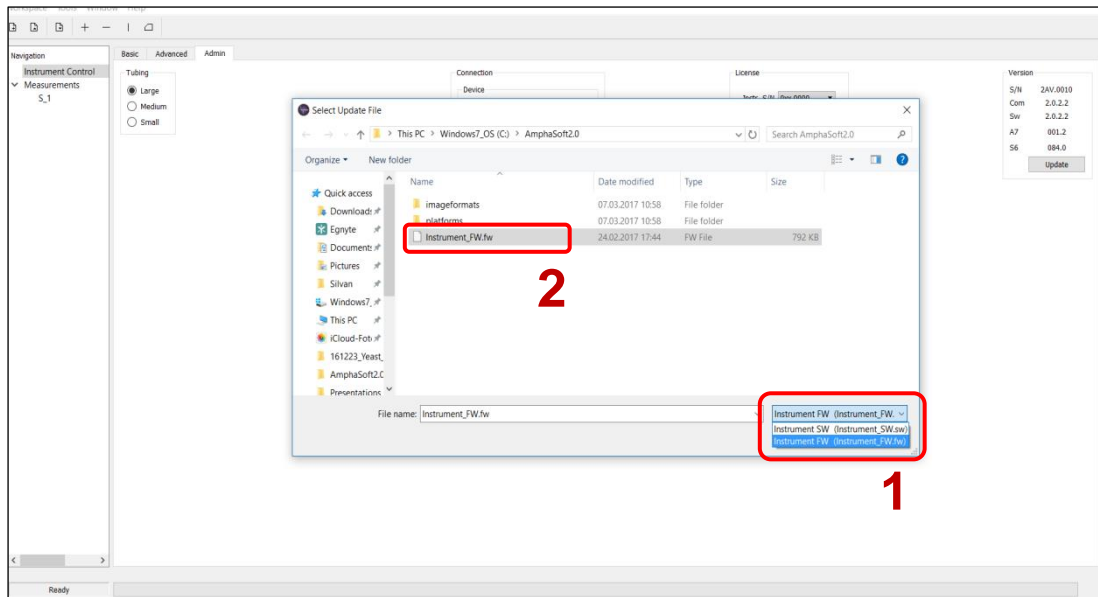
- Confirm the message by clicking *OK*
- Switch off the instrument, wait 5 seconds, and switch it on again.
- In case you receive the message "No connection to instrument", confirm the message by clicking *OK*
- Exit AmphaSoft 2.0
- After the *Instrument Software* update, you may have to update the *Instrument Firmware*. Reopen AmphaSoft 2.0 and wait 15 seconds before proceeding.
- Connect to the instrument in the *Admin* tab using the *Connect* button
- In case you need to do a firmware update, you will be notified with a popup window (see image below).



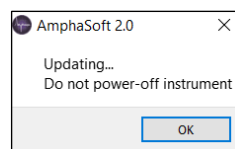
- Confirm the message by clicking *OK*
- On the **right hand side** of the *Admin* tab is the *Version* section (see image below).
- Click on *Update*



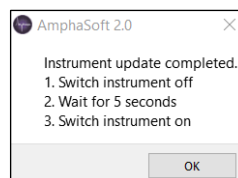
- In the new window, select **Instrument\_FW [1]** from the dropdown menu in the bottom right corner of the new window (see step 1 in the image above)
- Select the file **Instrument\_FW.fw [2]** in the window (see step 2 in the image above).
- Click *Open*



- There will be a reminder not to switch-off the instrument during the process (see image below).




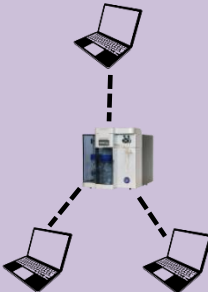

- The update process will take a few minutes.
- You will be notified about the completion with a popup window (see image below).



- Confirm the message by clicking **OK**
- Switch off the instrument, wait 5 seconds, and switch it on again.
- In case you receive the message “No connection to instrument”, confirm the message by clicking **OK**
- Exit AmphaSoft 2.0.
- Reopen AmphaSoft 2.0 and wait 15 seconds before proceeding.
- Connect to the instrument using the **Connect** button in the **Admin** tab. The update process is now completed.

## 9 Licensing

Amphasys offers several licensing models. Licenses are available in the form of a data file. There is a license file for the user licenses (standard, professional or AmphaFlex) and a measurement license (limited or unlimited).

License type	Standard	Professional	AmphaFlex
Operation modes	Online	Online and Offline	Online and Offline
Number of licenses	1	3	1 (+)
Number of measurements	unlimited	unlimited	limited
Scheme			

### 9.1 Uploading a License

If your instrument does not have any more available licenses, you can obtain additional licenses from Amphasys. Please contact your sales representative.

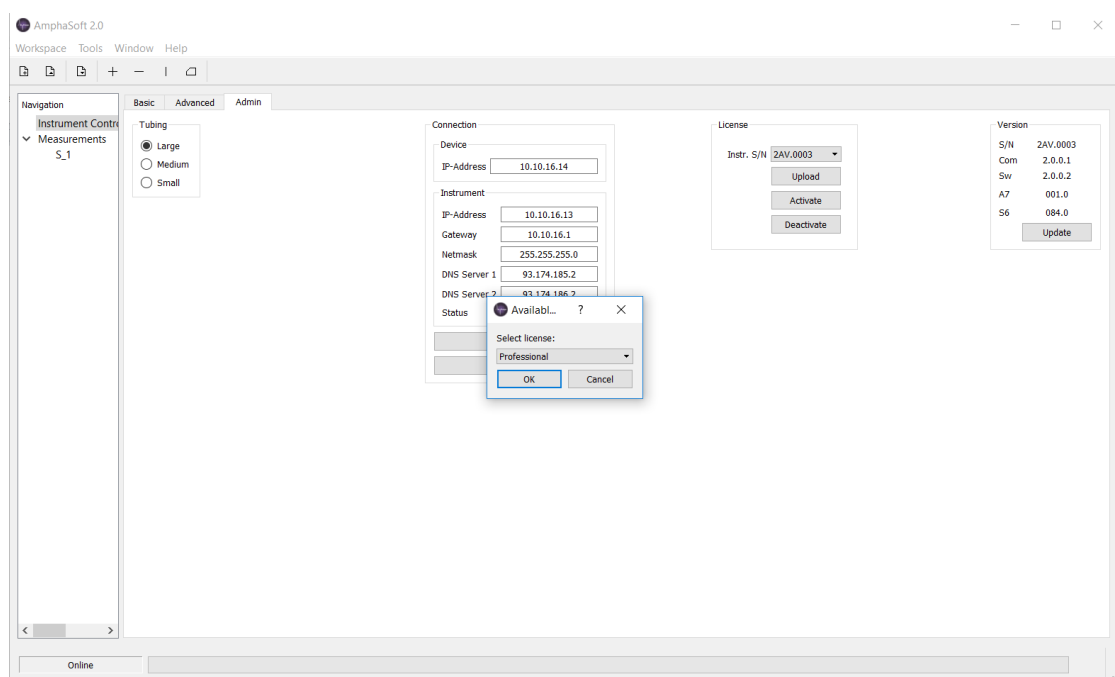
- After receiving a license, this license has to be uploaded to the instrument
- Start AmphaSoft 2.0 and connect to your instrument
- In the *License* section of the *Admin* tab, click *Upload*
- Select your license and click *Open*
- A popup informs you when the license installation succeeded.

## 9.2 Activation of a License

If your instrument has available user licenses (standard or professional), you can obtain a license for your computer using the following steps:

- Start AmphaSoft 2.0 and connect to your instrument
- In the *License* section of the *Admin* tab, click *Activate*
- Select the license from the dropdown-menu and click *Ok*

**Note** In case there is no available license, another license can be deactivated, or a new license can be purchased. Please be aware that deactivating removes a license from another user of the instrument. The number of deactivations and activations are limited.



## 9.3 Deactivation of a License

In case there is no available license, or a user is not using the license anymore (e.g. broken laptop, new laptop...), this license can be deactivated. A deactivated license can then be reactivated by another user. In order to deactivate a license, follow the steps below:

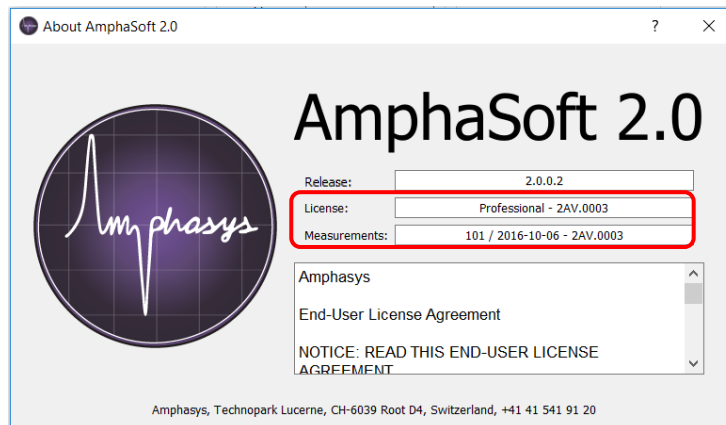
- Start AmphaSoft 2.0 and connect to your instrument
- In the *License* section of the *Admin* tab, click *Deactivate*
- Select the license from the dropdown-menu and click *OK*
- The number of remaining deactivations will be shown

**Note** The number of deactivations is limited.

## 9.4 AmphaFlex

For the AmphaFlex license model the number of measurements and/or the time of using the instrument is limited, e.g. 1 year with 1000 measurements. An upgrade to unlimited licenses or a higher number of measurements or longer time frame is always possible.

When connected to the instrument, you can get your current licensing status under *Help > About*. The number of available measurements and the license expiration date are shown.



## 10 Troubleshooting

### Connection to instrument not possible

- Make sure the ethernet cable (with or without the USB adapter) is correctly plugged to instrument and PC. If yes, quickly unplug and plug the cable or adapter. Try to connect again.
- Power-cycle your instrument and close AmphaSoft 2.0. Start AmphaSoft 2.0 again and try to connect.
- If the connection fails, check the Ethernet port configuration using the *Ampha Z32 User Guide*. It contains a section about configuring the Ethernet port with or without USB adapter.
- Prevent USB / Ethernet ports from going into sleep-mode

For other issues please have a look at our *Troubleshooting Quick Guide* on our website or contact Amphasys support.



## 11 Warranty and Technical Support

### 11.1 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 <https://amphasys.com/>.

### 11.2 Extended Warranty

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

### 11.3 Support

For technical or software support, please contact

**Amphasys AG**

Technopark Lucerne

Platz 4

CH-6039 Root D4, Switzerland

Phone: +41 41 541 91 20

Email: [support@amphasys.com](mailto:support@amphasys.com)