



... Reinventing Single
Cell Analysis

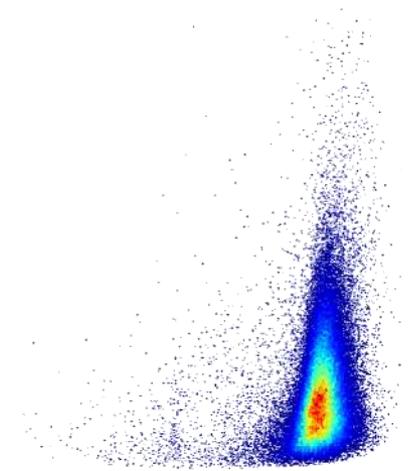
Amphasys Introduction



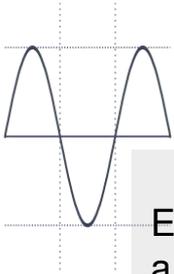
Contents



- Introduction to Impedance Flow Cytometry
- From sample to result
 - Measurement principles
 - Lab-on-chip technology
- Measurement procedure
- Summary
- Contact and useful links



Impedance Flow Cytometry (IFC)

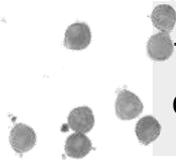


Electrical **Impedance** is opposition to a current in a circuit when an alternating voltage is applied

The cells are suspended in a conductive **Fluid**

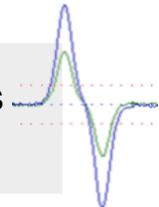


Impedance Flow Cytometry

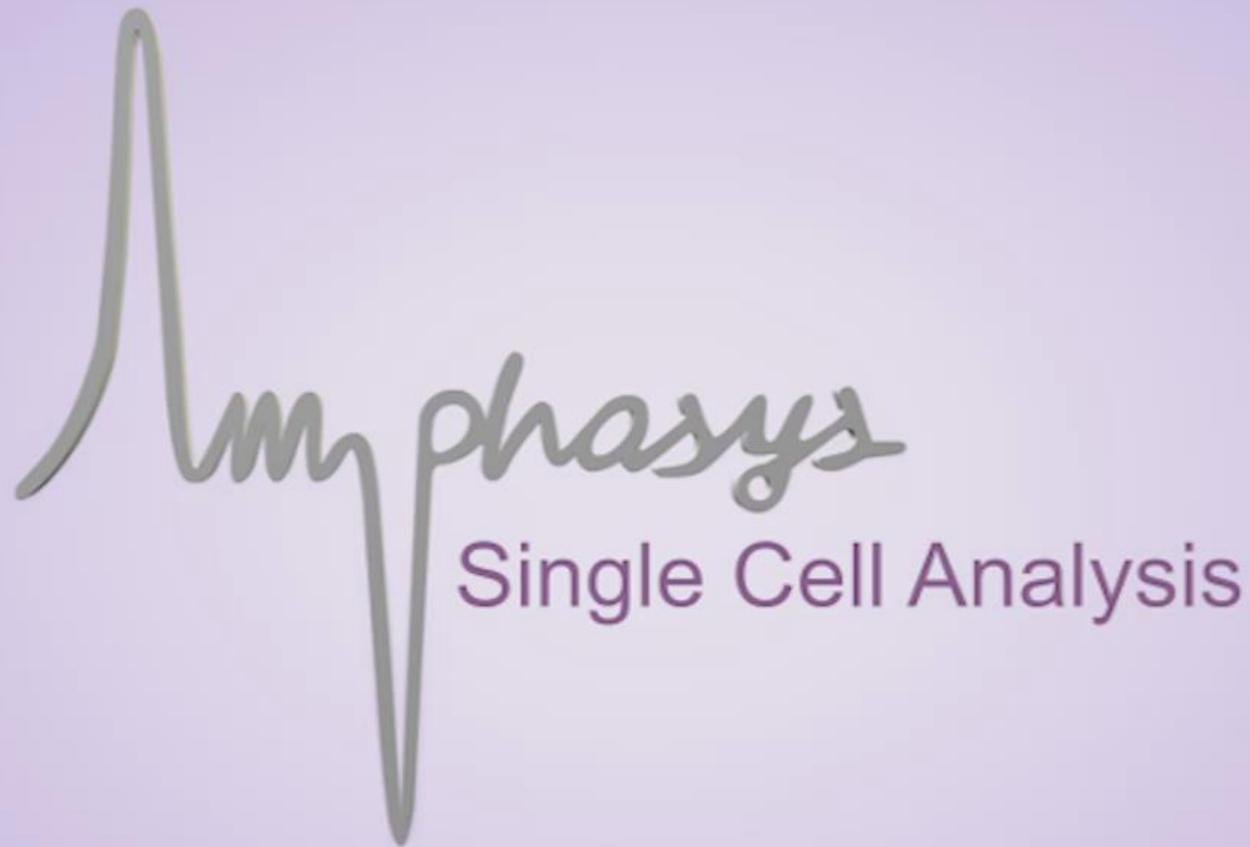


The technology is used to characterize **Cells**

Electrical properties of cells are **Measured**



Impedance Flow Cytometry (IFC)

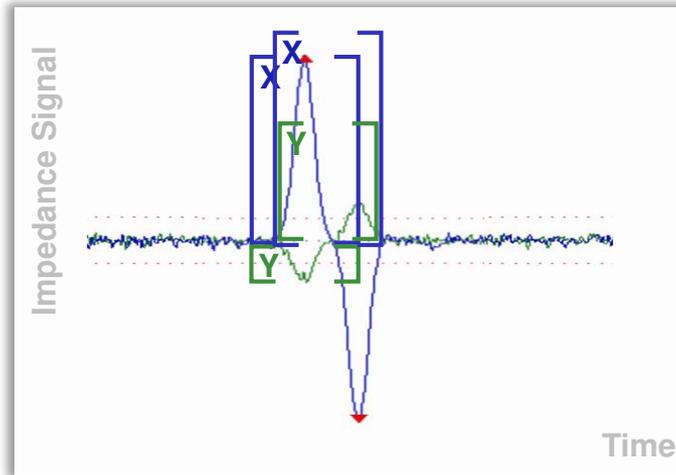


PRtools

How we measure cells...



- Viable Cell
- Dead Cell



- Real Part (X)
- Imaginary Part (Y)
- Peak Positions
- Triggering Levels

Sample



Chip



Microelectrodes

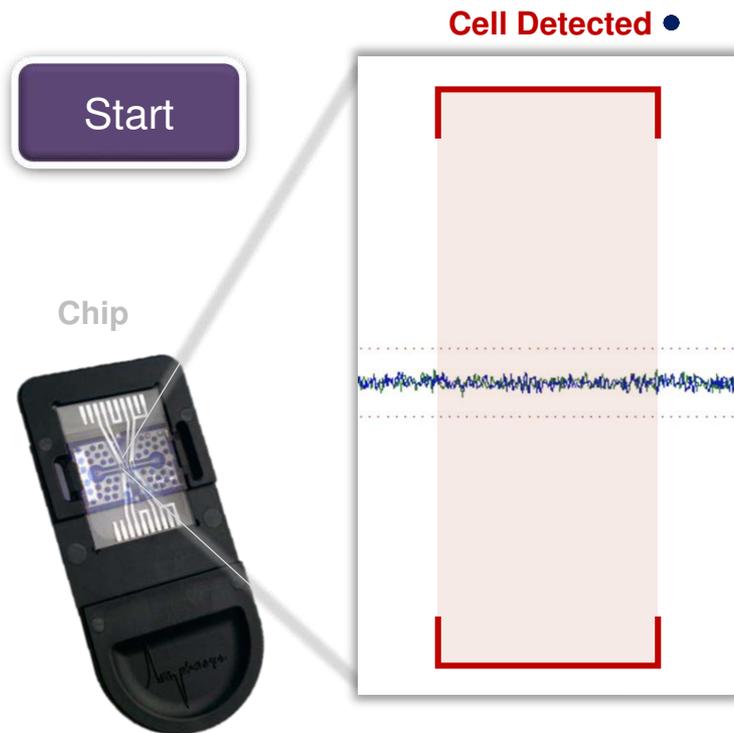


Microchannel

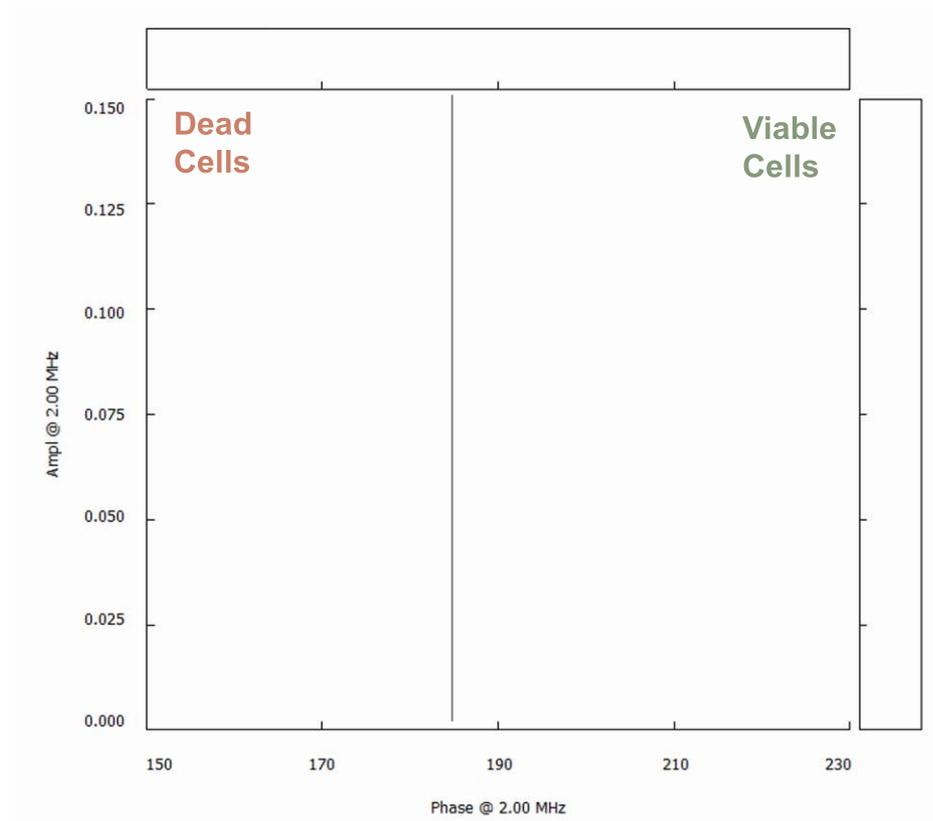
How we measure cells...



Impedance Signals



Phase – Amplitude Scatterplot



...lots of cells



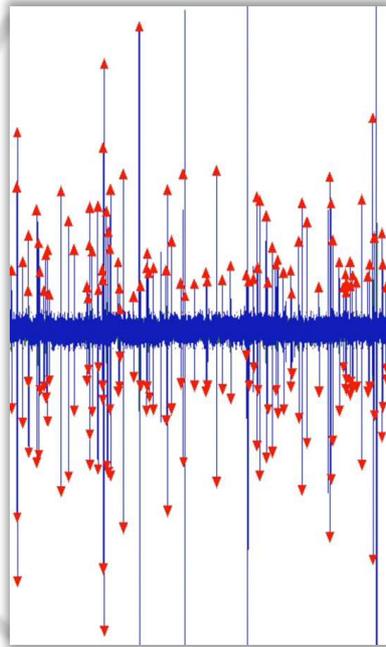
Impedance Signals

Start

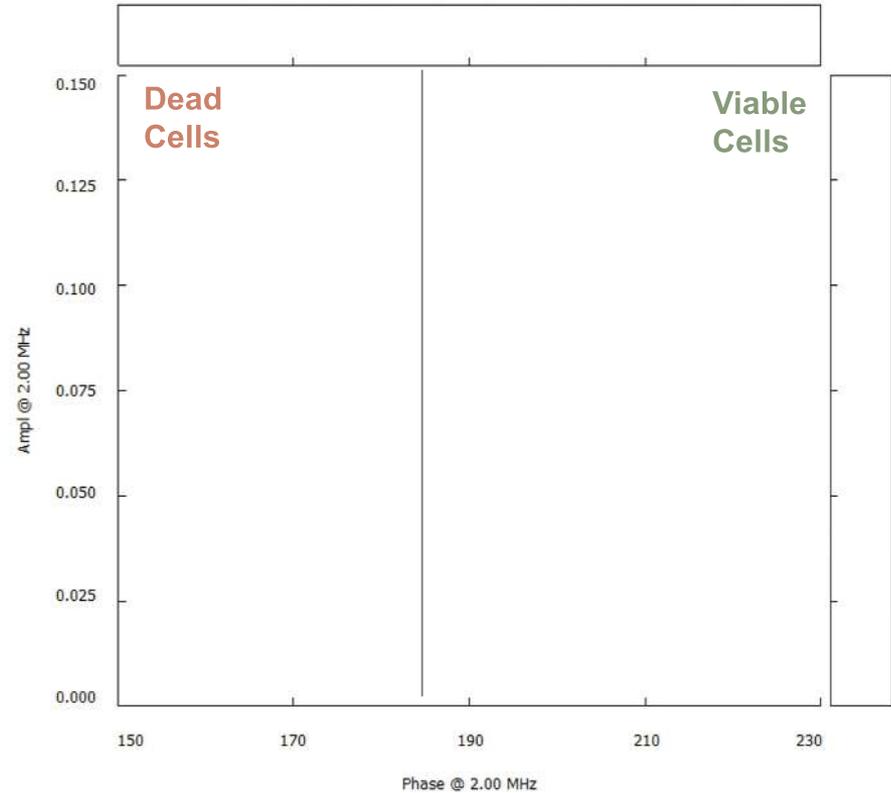
Chip



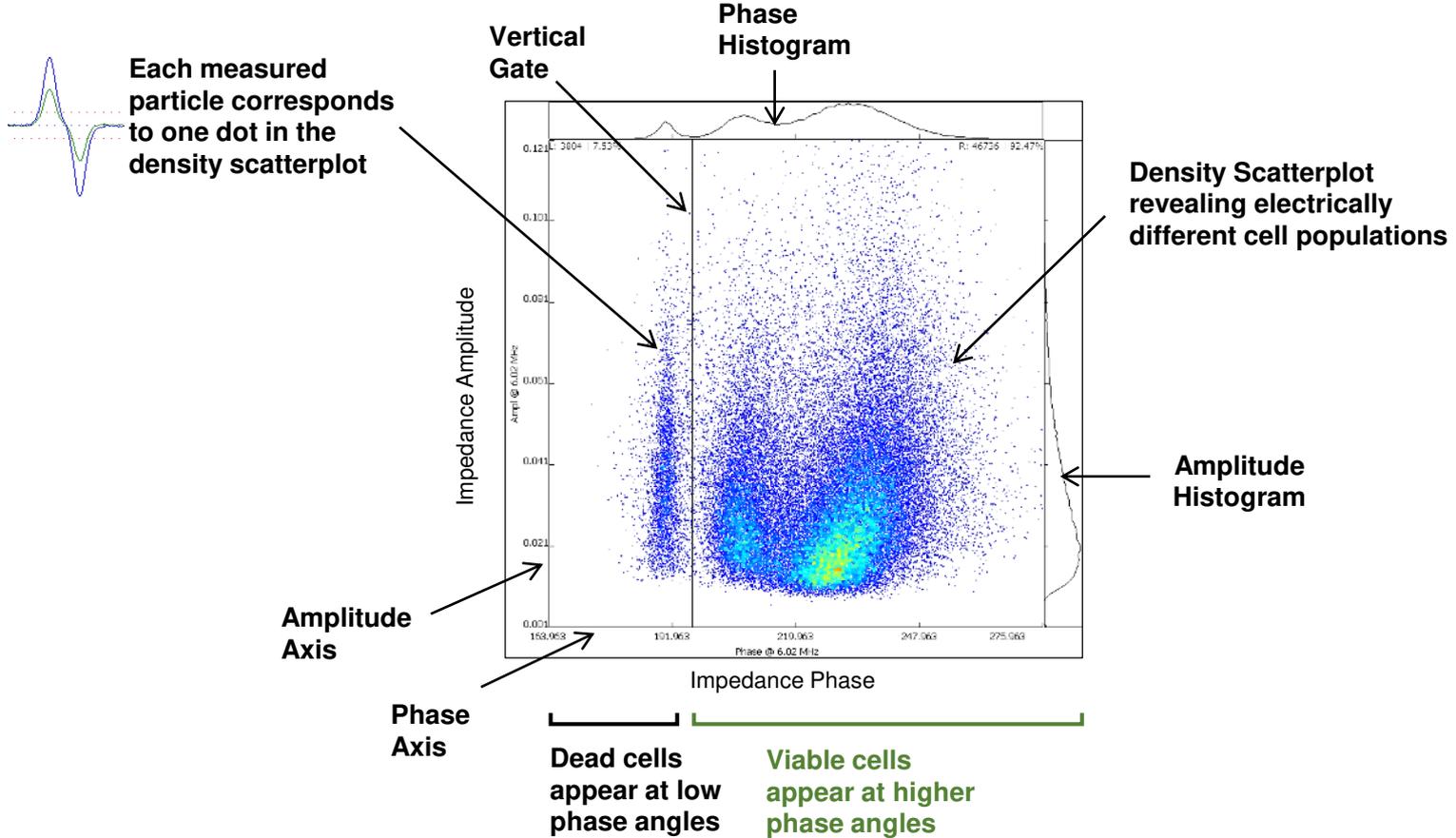
Real-time cell analysis



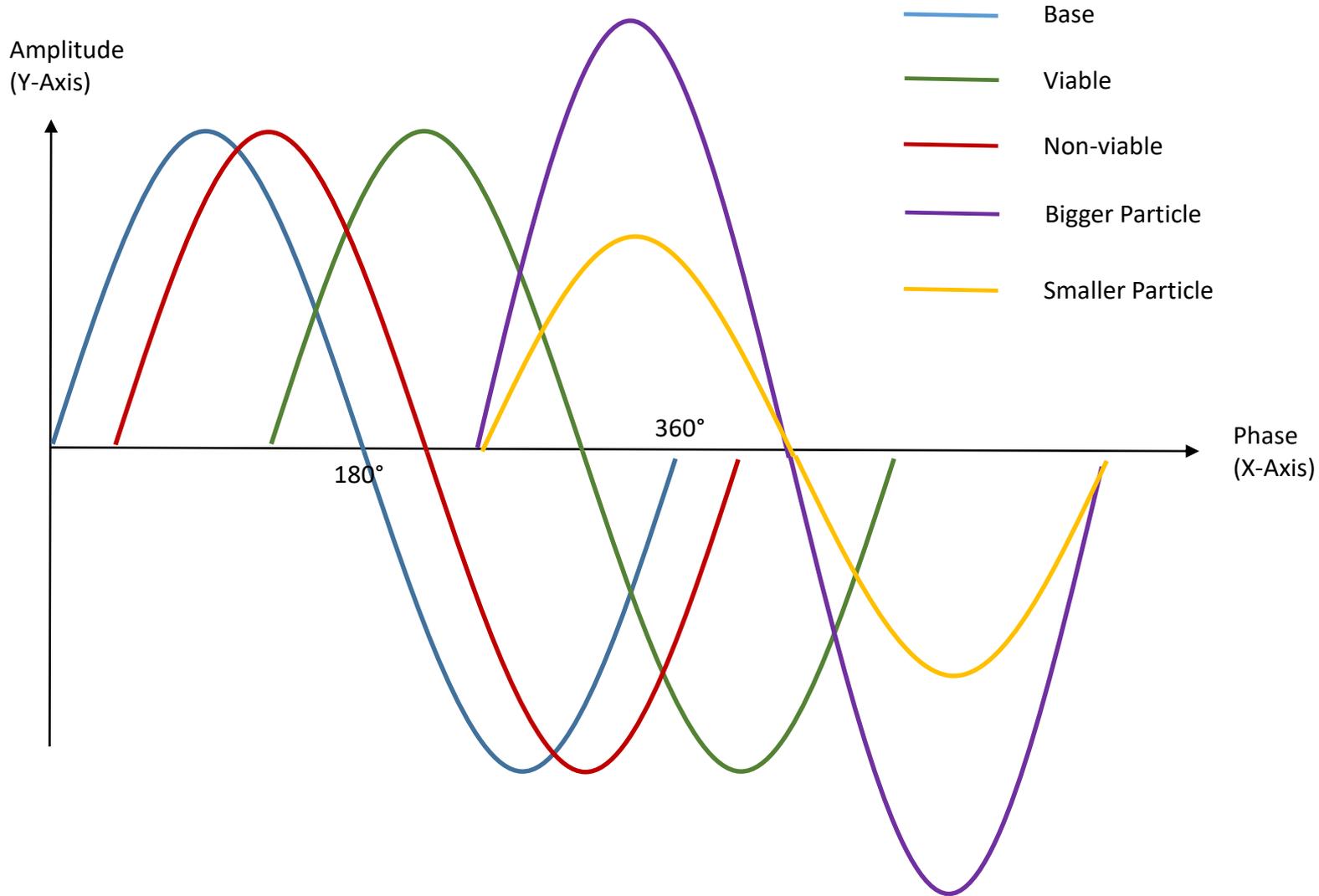
Phase – Amplitude Scatterplot



What do we see in a scatterplot?



Understanding the axis



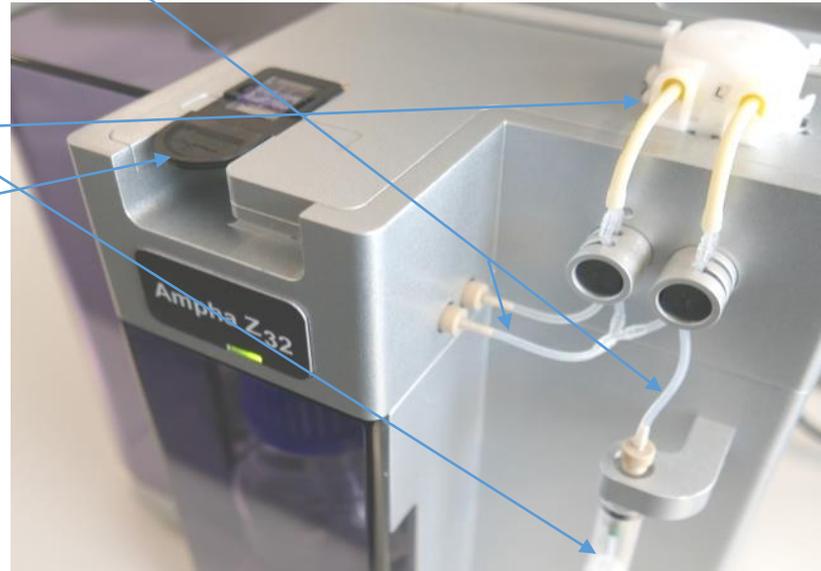
AmphaZ32 System – Where to find what



AmphaZ32 System - Fluidics



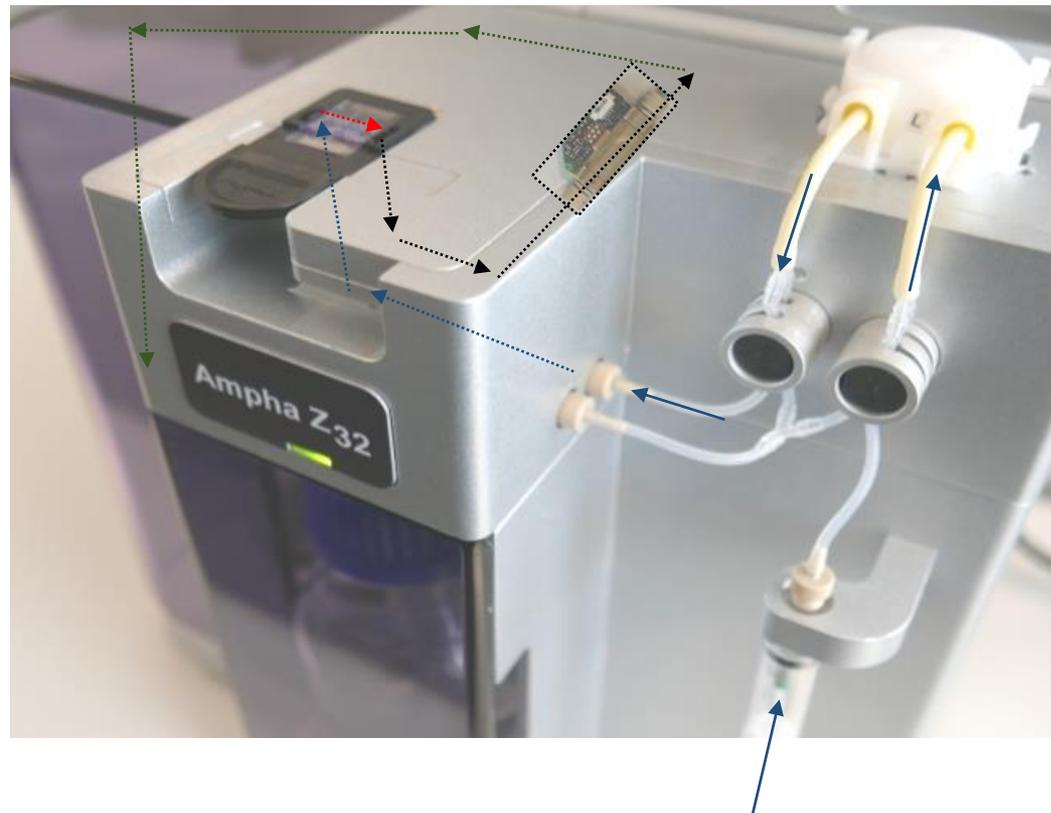
- Silicone and Teflon tubing system which transports the sample
- Sample tube
- Peristaltic pump
- Chip
- Flow sensor
- Waste



AmphaZ32 System – The sample pathway



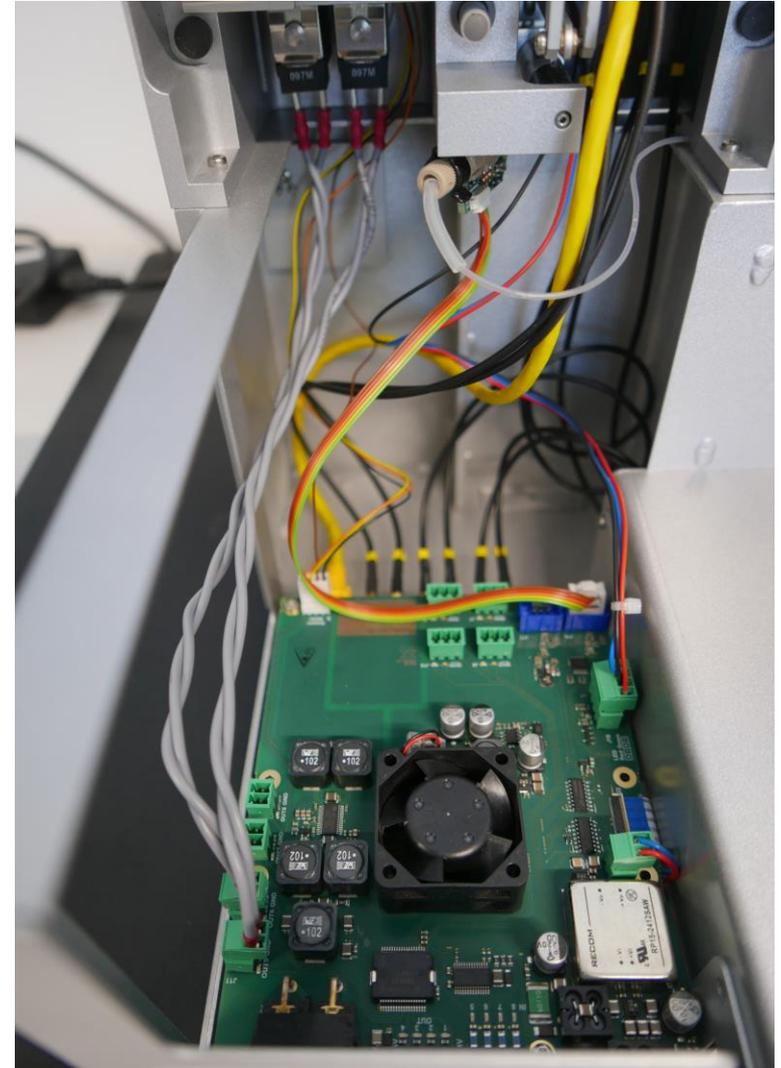
- Sample aspiration
- **Measurement**
- Flow Meter
- Waste bottle



AmphaZ32 System - Electronics



- **Periphery Board**
- Pinch valves
- Stepper Motor
- Front-LED
- Flow Sensor
- Power Switch
- Power cable (for Control Board)
- Coax cables (connected to Control Board)



AmphaZ32 System - Electronics



- Periphery Board
- Control Board
- Sensor Driver Board
- Sensor Receiver Board

AmphaZ32 System – Amphasys Laptop



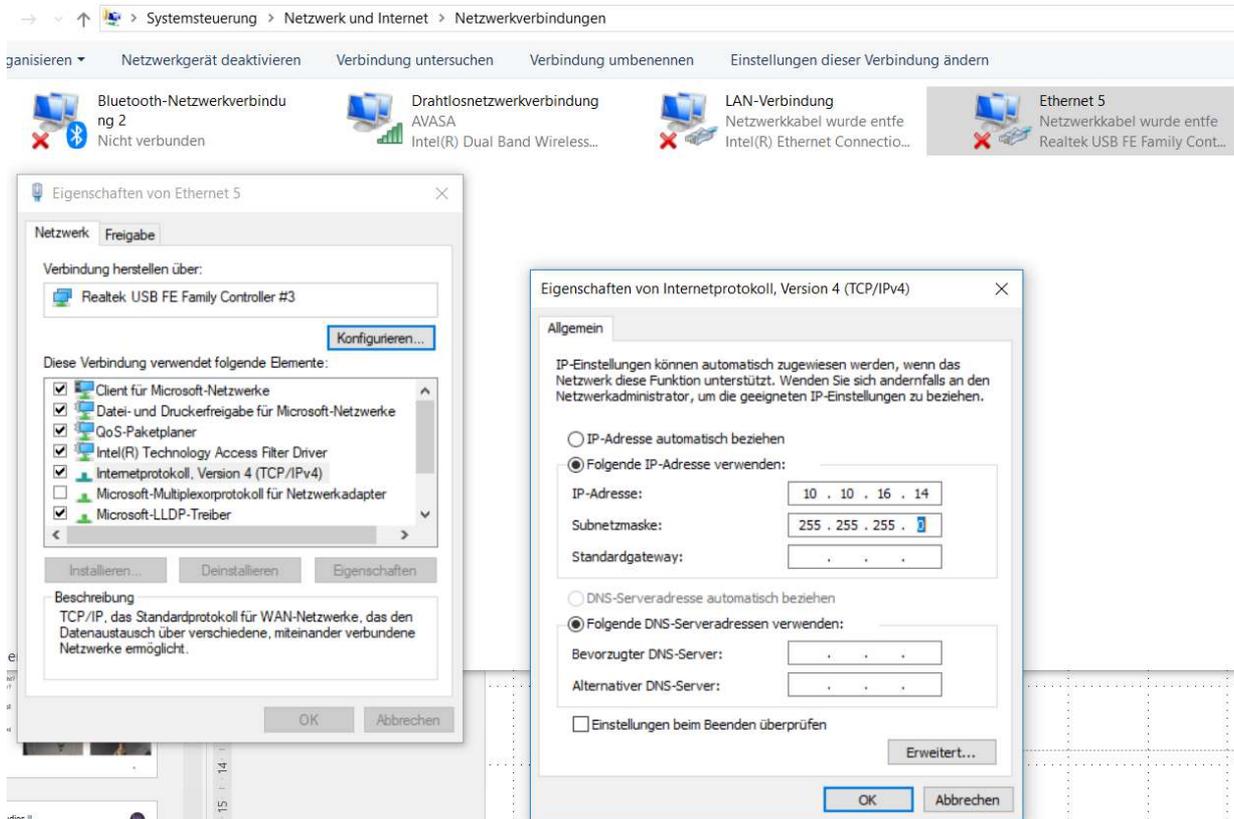
- Buying from Amphasys makes your life easier !!! (20% of our Support cases are dealing with „self-bought“ customer laptops/ computers)
- Meets all minimum requirements
 - i5 64 bit dual core processor or higher (recommended: i7)
 - 8 GB RAM
 - At least 128 GB SSD (recommended: 256 GB SSD)
 - Full HD screen (screen resolution 1920x1080, recommended: 15' screen)
 - Windows 7 or 10 Pro (ASCII)
 - OpenGL 3.3 graphics card or higher
 - At least 2 free USB 2.0 connectors
 - Internet access (WLAN or Ethernet)
 - USB-Mouse
- Comes pre-configured



AmphaZ32 System – Amphasys Laptop



- Comes pre-configured
- USB-to-Ethernet Adapter
- Needs to be configured



AmphaZ32 System – Licensing



- License types
 - **User licenses**
 - Professional
 - Typically in a bundle of 3 licenses
 - Offline and online operation mode
 - Advanced data analysis options
 - Standard
 - Typically a single license
 - Online operation mode only
 - No advanced data analysis options
 - **Measurement licenses**
 - Unlimited
 - Measurement and/or time-limited
- Stored in Instrument
- Connected laptop/ computer can obtain license
 - 1 Activation = -1 License on Instrument



AmphaZ32 System - Chips



▪ Measurement Chips

- Letter/ Channel size coded
- (A – 15 μ m) B – 30 μ m, C – 50 μ m, F – 80 μ m, D – 120 μ m, E – 250 μ m
- Choice of Chip very important for succesful measurement



▪ Calibration Chip

- Defined Resistance, For Maintenance/ Support only
- Calib1 – 12kOhm, Calib2 – 30kOhm

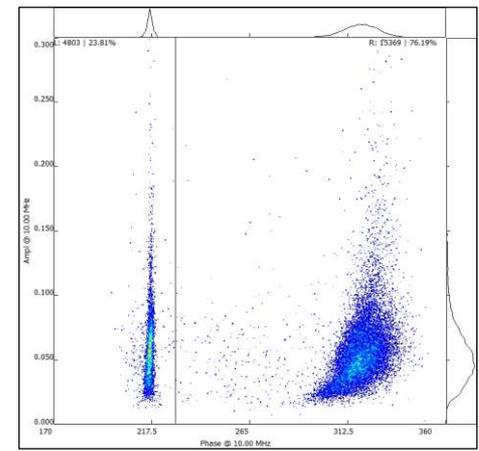


▪ Cleaning Chip

- Mandatory for cleaning with Ethanol
- Optional for cleaning with AmphaClean



From Sample to Result



From Sample to Result



- Successful measurement require good preparation of settings
 - Garbage in → Garbage out
- [Recommendation list for buffers, filters, chips and settings](#)

AmphaFluid Buffer List

June 2018



The following AmphaFluid (AF) buffers for measurement of pollen (po) or microspores (msp) are best practise suggestions. They are most likely to produce good results for viability, development stages and ploidy measurements. It is recommended to use fresh samples and to filter the samples before use and measure immediately. Dried pollen samples should be rehydrated before measurement. Pollen and microspores are complex particles influenced by many factors that can have an effect on the outcome of the measurements. Therefore, the use of AF buffers is no guarantee for results.

Overview	Typical buffer recommendation and other information
Monocotyledonae	AF6
cereals (e.g. wheat, rye, barley)	E-chip 250 µm, attention: short pollen life time, pollen may burst
AF buffer recommendations	for pollen viability: column "AF buffer pollen" for microspores: column "AF buffer microspore" for other purposes: try "alternative AF buffers", too
sticky pollen	add 0.05 to 0.1% Tween 20 or 80

Recommendations for chips, filters and settings for Ampha Z30 or Z32

Pollen size	AmphaChip	Filter	Trigger	Frequency	Modulat.	Amplificat.	Demodulat.	
10-20 µm	F 80 µm	30/50 µm	0.05 x+	2 MHz	4	6	1 or 2	(or: 5-5-0)
20-30 µm	F 80 µm	50 µm	0.05 or 0.1 x+	2 MHz (0.5 MHz y-)	3	6	2	
20-30 µm	D 120 µm	50 µm	0.05 or 0.1 x+	2 MHz (0.5 MHz y-)	4	6	1 or 2	
30-40 µm	D 120 µm	50 µm	0.1 x+	2 MHz (0.5 MHz y-)	3	6	1 or 2	
40-50 µm	D 120 µm	70 µm (100 µm low concl)	0.1 x+	1 MHz (0.5 MHz y-)	3	6	1	
40-70 µm	E 250 µm	100 µm	0.1 x+	2 MHz (0.5 MHz y-)	3	6	2	
70-90 µm	E 250 µm	100 µm	0.1 x+	2 MHz (0.5 MHz y-)	3	6	1	
90-130 µm	E 250 µm	150 µm	0.2 x+	2 MHz (0.5 MHz y-)	3	6	0	
130-160 µm	E 250 µm	200 µm (low concl) *	0.2 x+	2 MHz (0.5 MHz y-)	2	6	0	

Species	English	German	Family	Class	Size (µm)	Form	AF buffer pollen	AF buffer microspores	alternative AF buffers
Acer campestre	field maple	Feld-Ahorn	Sapindaceae	Dicotyledonae	26	triangular	6		5
Acer platanoides	sycamore maple	Berg-Ahorn	Sapindaceae	Dicotyledonae	32	triangular	6		5
Acer pseudoplatanus	norway maple	Spitz-Ahorn	Sapindaceae	Dicotyledonae	38	triangular	6		5
Actinidia deliciosa	kiwi fruit	Kiwi	Actinidiaceae	Dicotyledonae	40	triangular	5		6
Aesculus hippocastanum	buckeye	Roskastanie	Sapindaceae	Dicotyledonae	24	round	6		5
Aesculus pavia	red buckeye	Roskastanie	Sapindaceae	Dicotyledonae	26	round	6		5
Agapanthus	lily of the Nile	Schmucklilie	Amaryllidaceae	Dicotyledonae	55	prolate	6		5
Alcea rosea	hollyhock	Stockrose	Astereaceae	Dicotyledonae	110	round spiny	6		4 / 2
Allium cepa	onion	Zwiebel	Liliaceae	Monocotyledonae	30	prolate	6		4
Allium giganteum	giant onion	Zierlauch	Liliaceae	Monocotyledonae	35	prolate	6		4
Allium porrum	leek	Lauch	Liliaceae	Monocotyledonae	30	prolate	6		4
Allium schoenoprasum	chive	Schnittlauch	Liliaceae	Monocotyledonae	26	prolate	6		4
Allium ursinum	wild garlic	Bärlauch	Liliaceae	Monocotyledonae	30	prolate	6		4

From Sample to Result - Sample Preparation



- Work with standardized protocols if you want to compare results
 - Buffer, Chip Type, Settings, Filter Size, Sample Preparation Methodology
- Know the stability of the cells in the buffer
- Add 0.05 % Tween 20 to the buffer aliquot for hydrophobic cells
- Treat your cells gently
 - Do not forcefully squeeze anthers with a pistil in order to release pollen
- Prepare the sample as clean as possible
 - Minimize the amount of debris
 - Use cells in the most pure form without other confounding particles, dust, debris...
- Check the buffer quality
 - Clear solution, no microbial growth
 - Buffer equilibrated to room temperature
- Use appropriate cell concentrations to minimize the risk of chip clogging while still being able to acquire high cell numbers in a short time





- Note: AmphaSoft 2.0 has default instrument settings, which work fine for 95 % of the applications (given an optimal sampling and sample preparation method according to the Amphasys recommendations).
- The AmphaSoft default settings are chip type specific and robust. They should cover variations of the following factors:
 - Chip Type (Amphasys Chips)
 - Buffer Composition (AmphaFluid Buffers)
 - Hydration Status of the Cell
 - Residence Time in Buffer
- In case an application does not yield satisfactory results, it may be worthwhile optimizing parameters, e.g. the measurement frequency. This is an advanced task, as changing one parameter may require adjusting multiple other parameters. For experienced users, optimizing settings is straightforward. But it requires a good understanding of all measurement parameters and their implications on each other.

From Sample to Result - Experimental Setup



- The experimental setup mostly aims at providing a good resolution between cell subpopulations, e.g. viable and dead cells

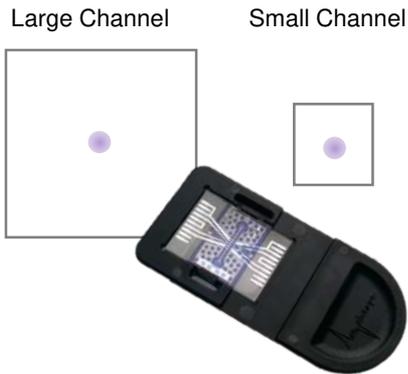
- Factors affecting the discrimination between cellular subpopulations as previously mentioned are:
 - **Chip Type (very important)**
 - *Better discrimination for higher particle-to-channel size ratios*
 - **Buffer Composition (very important)**
 - *The buffer affects signal-to-noise ratio, resolution of populations and the optimum analysis frequency*
 - **Measurement Frequency (important)**
 - *Optimum frequency depends on application, e.g. viability or ploidy*
 - Hydration status of the cell, e.g. after storage (sometimes important)
 - *Dehydrated and frozen cells sometimes behave like dead cells*
 - Residence time in buffer (not so important)
 - *Electrical properties of cells change with increasing residence time*

From Sample to Result - Resolution between cell subpopulations



Chip

Cell-to-channel size ratio



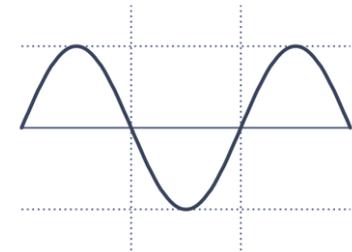
Buffer

Composition



Electric Field

Frequency



- Higher cell-to-channel size ratios result in higher signal-to-noise and better resolution between cell subpopulations
- Higher cell-to-channel size ratios also increase the risk of chip clogging
- Chip type depends on cell diameter (is given for an application)
- Default instrument settings are chip type specific (typically no need for optimizations)

- The buffer composition influences the signal-to-noise ratio and the resolution between cell subpopulations
- The optimum analysis frequency depends on the buffer composition
- Default instrument settings are robust for the use of any Amphasys buffer

- The signal-to-noise ratio decreases for increasing numbers of frequencies simultaneously applied
- 2 frequencies are recommended for most applications
- Changing the triggering frequency (by default the first frequency) typically requires adaptation of other parameters as well (Triggering source, Triggering direction, Level). Only adjust the triggering frequency if necessary.

From Sample to Result – AmphaSoft Structure



The screenshot displays the AmphaSoft software interface. At the top, the title bar reads "AmphaSoft" and the menu bar includes "Workspace", "Tools", "Window", and "Help". The main window is titled "Main menu".

On the left side, there is a "Navigation" panel (highlighted with an orange border) containing "Instrument Control" and "Measurements" (with "S_1" selected). A vertical orange label "Navigation" is positioned to the left of this panel.

The central area is the "Measurement" tab (highlighted with a green border). It features a "Settings" section with fields for "Sample" (Id: S_1, Name:), "Buffer" (Id: AF2), "Chip" (Id: D00000), and "Frequency [MHz]" (1: 0.50, 2: 12.00, 3: 0.00, 4: 0.00). Below this is the "Stop Conditions" section (Cells: 0, ul: 0, min:sec: 0:0) and the "Axis" section (Freq: 1, Phase: 0.00, Ampl: 0.00, Shift: 0.00). A "Reset" button is located at the bottom of the settings.

Two plots are displayed side-by-side. The left plot is titled "Ampl @ 0.50 MHz" and the right plot is "Ampl @ 12.00 MHz". Both plots show a single sharp peak at approximately 360 degrees phase. A green label "Measurement tab" is centered over the plots.

At the bottom of the measurement area, there is a "Results" section with fields for "Concentration [cells/ml]", "Average Flow [ul/min]", and "Duration". To the right of these fields are "Accepted" and "Rejected [%]" indicators. A "Start Measurement" button is located at the bottom center of the measurement area.

At the very bottom of the interface, there is a "Status section + Main button" (highlighted with an orange border) showing "Offline" on the left and "0%" on the right.

From Sample to Result - Worklist



AmphaSoft
Workspace Tools Window Help

Navigation
Instrument Control
Measurements
S_1

Worklist

	Id	Sample Name	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Note	Done
1	S_1		AF1	B00000	0.5	12		0

Set... ?

- Measurement Id
- Sample Name
- Buffer Id
- Chip Id
- Freq 1
- Freq 2
- Freq 3
- Freq 4
- Stop Cond Cells
- Stop Cond UI
- Stop Cond Min

Select All

OK
Cancel

Measurements + - 1 Settings +/-

Report Layout Comparison

Offline 0%

Additional Settings

From Sample to Result - Worklist



- Always confirm new configurations (Sample name, Chip name, Buffer ID, Stop Conditions...) with [Enter]

The screenshot shows the AmphaSoft 2.0 software interface. The title bar reads "AmphaSoft 2.0". Below it is a menu bar with "Workspace", "Tools", "Window", and "Help". A toolbar contains icons for file operations and window management. On the left is a "Navigation" pane with "Instrument Control" and "Measurements" (expanded to show "S_1 - Sample 1"). The main area is titled "Worklist" and contains a table with the following data:

	Id	Sample Name	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Note	Done
1	S_1	Sample 1	AF6	D12345	2	12		0

From Sample to Result - Reporting



AmphaSoft 2.0 - 150928_ornamentals-AmphaSoft2.0

Workspace Tools Window Help

Navigation

Instrument Control

- Measurements
 - S_1: 8'207 | 26'923
 - S_2: 4'954 | 28'319
 - S_3: 5'200 | 22'366
 - S_4: 5'678 | 25'097
 - S_5: 8'178 | 63'614
 - S_6: 7'413 | 31'335
 - S_7: 7'978 | 37'722
 - S_8: 12'931 | 40'568
 - S_9: 2'398 | 5'429
 - S_10: 2'054 | 4'185
 - S_11: 820 | 732
 - S_12: 1'961 | 2'127
 - S_13: 10'168 | 64'812

Worklist

	Id	Sample Name	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Note	Done
1	S_1	delosperma pink	AF5	D00283	0.5	12		1
2	S_2	delosperma pink	AF5	D00283	0.5	12		1
3	S_3	delosperma pink	AF5	D00283	0.5	12		1
4	S_4	delosperma pink	AF6	D00283	0.5	12		1
5	S_5	delosperma gelb	AF5	D00283	0.5	12		1
6	S_6	delosperma gelb	AF6	D00283	0.5	12		1
7	S_7	delosperma weiss	AF5	D00283	0.5	12		1
8	S_8	delosperma weiss	AF6	D00283	0.5	12		1
9	S_9	campanula	AF5	D00283	0.5	12	old flower	1
10	S_10	campanula	AF6	D00283	0.5	12	old flower	1
11	S_11	armeria grasnelke	AF5	D00283	0.5	12	old	1
12	S_12	armeria grasnelke	AF6	D00283	0.5	12	old	1
13	S_13	oil palm	AF6	D00283	0.5	12		1

Measurements + - 1 Settings +/-

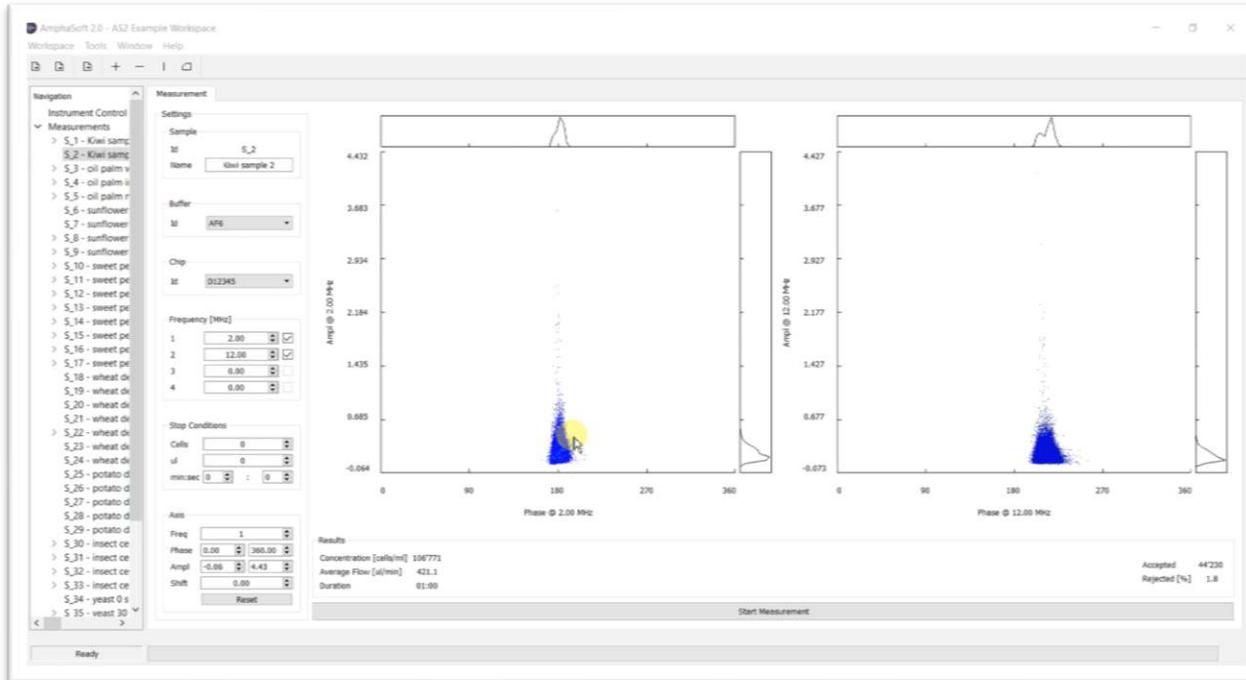
Report Layout Comparison

Offline 0%

From Sample to Result - Data Visualization



Video: Appropriate data visualization using zoom, stretch, point size and density coloring.



Move clouds

- Click and drag

Zoom

- Mouse Wheel
- Define *phase* and *amplitude* axes limits

Dot size

- Ctrl + mouse wheel

Density coloring

- Alt + mouse wheel

Phase lower axis limit

Amplitude lower axis limit



Axis

Freq

Phase

Ampl

Shift

Plot number

Phase upper axis limit

Amplitude upper axis limit

Artificial phase shift

Go to default axis limits

From Sample to Result - Reporting



AmphaSoft 2.0 - 150928_ornamentals-AmphaSoft2.0

Workspace Tools Window Help

Navigation

Instrument Control

- Measurements
 - S_1: 8'207 | 26'923
 - S_2: 4'954 | 28'319
 - S_3: 5'200 | 22'366
 - S_4: 5'678 | 25'097
 - S_5: 8'178 | 63'614
 - S_6: 7'413 | 31'335
 - S_7: 7'978 | 37'722
 - S_8: 12'931 | 40'568
 - S_9: 2'398 | 5'429
 - S_10: 2'054 | 4'185
 - S_11: 820 | 732
 - S_12: 1'961 | 2'127
 - S_13: 10'168 | 64'812

Worklist

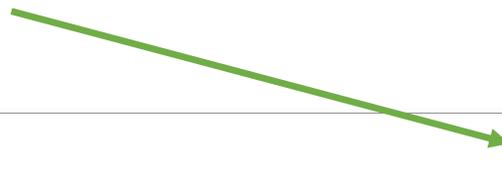
	Id	Sample Name	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Note	Done
1	S_1	delosperma pink	AF5	D00283	0.5	12		1
2	S_2	delosperma pink	AF5	D00283	0.5	12		1
3	S_3	delosperma pink	AF5	D00283	0.5	12		1
4	S_4	delosperma pink	AF6	D00283	0.5	12		1
5	S_5	delosperma gelb	AF5	D00283	0.5	12		1
6	S_6	delosperma gelb	AF6	D00283	0.5	12		1
7	S_7	delosperma weiss	AF5	D00283	0.5	12		1
8	S_8	delosperma weiss	AF6	D00283	0.5	12		1
9	S_9	campanula	AF5	D00283	0.5	12	old flower	1
10	S_10	campanula	AF6	D00283	0.5	12	old flower	1
11	S_11	armeria grasnelke	AF5	D00283	0.5	12	old	1
12	S_12	armeria grasnelke	AF6	D00283	0.5	12	old	1
13	S_13	oil palm	AF6	D00283	0.5	12		1

Measurements + - 1 Settings +/-

Report Layout Comparison

Offline 0%

Create your reports here



From sample to Result - Reports



- .csv or .html-file
- Comparison or Measurement sorted

report.csv - Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW PDF Architect 4 Creator

Calibri 11 Font: Bold, Italic, Underline, Text Color, Background Color, Paragraph: Bullets, Numbered, Indent, Decrease Indent, Increase Indent, Merge & Center, Alignment: Left, Center, Right, Justify, Wrap Text, Number: Percentage, Increase Decrease, Conditional Formatting, Format as Table, Check Cell

A4 Date/Time: Wednesday 05-Sep-2018 08:54

AmphaSoft Measurement Report													
SW Version: 2.1.2.0													
Workspace:													
Date/Time: Wednesday 05-Sep-2018 08:54													
Settings													
Id	Sample N	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Trig Level [0.0001-20V]	Mod [1-5]	Ampl [1-8]	Dem [0-8]	Note	Done	Report	
S_1	Wheat via	AmphaCalib	E00231	2	12	0.1	3	6	2		0	1	
S_2	Wheat via	AF6	E00231	2	12	0.1	3	6	2		1	1	
S_3	Wheat via	AF6	D00342	2	12	0.05	3	6	1		1	1	
S_4	Wheat de	AF6	D00342	2	12	0.05	3	6	1		1	1	
S_5	Wheat mi	AmphaCalib	D00342	2	12	0.05	3	6	1		1	1	
S_6	Wheat mi	AF6	D00342	2	12	0.05	3	6	1		1	1	
S_7	Wheat de	AF6	D00342	2	12	0.05	3	6	1		0	1	
Results													
Id	Sample N	Date/Time	Duration[mm:ss]	Concentra	MeanFlow	MeasVol[ul]	Accepted	Rejected[%]					
S_2	Wheat via	09/05/2017 11:19	00:35	2054	2402.5	1403	2882	3.2					
S_3	Wheat via	09/05/2017 11:30	01:30	4725	473.5	711.6	3363	4.3					
S_4	Wheat de	09/05/2017 11:52	01:41	4069	483.1	814.6	3315	7.6					
S_5	Wheat mi	09/05/2017 11:38	01:43	10809	448.2	770.4	8328	4.1					
S_6	Wheat mi	09/05/2017 11:55	02:26	16319	480.5	1170.7	19105	4					
Gating Statistics													
Cross Gating @ 2.00 MHz													
Id	Sample N	UL-Count	UL[%]	UL[cells/n]	UR-Count	UR[%]	UR[cells/n]	LR-Count	LR[%]	LR[cells/n]	LL-Count	LL[%]	LL[cells/ml]
S_3	Wheat via	1115	33.15	1566	937	27.86	1316	927	27.56	1302	384	11.43	539
S_4	Wheat de	1249	37.68	1533	84	2.53	103	1452	43.8	1782	530	15.99	650
Vertical Gating @ 2.00 MHz													
Id	Sample N	L-Count	L[%]	L[cells/ml]	R-Count	R[%]	R[cells/ml]						
S_2	Wheat via	773	26.82	550	2109	73.18	1503						
Cross Gating @ 12.00 MHz													
Id	Sample N	UL-Count	UL[%]	UL[cells/n]	UR-Count	UR[%]	UR[cells/n]	LR-Count	LR[%]	LR[cells/n]	LL-Count	LL[%]	LL[cells/ml]
S_3	Wheat via	1180	35.09	1658	886	26.35	1245	931	27.68	1308	366	10.88	514
S_4	Wheat de	1228	37.04	1507	108	3.26	132	1649	49.74	2024	330	9.96	405



AmphaSoft 2.0 Measurement Report

SW Version: 2.1.2.0
 Workspace:
 Date/Time: Freitag 12-Jan-2018 11:58
 Measurements: [Settings](#) [Results](#) [Gating Statistics](#) [Gating Views](#)

Settings

Id	Sample Name	Buffer Id	Chip Id	Freq 1 [0.1-30MHz]	Freq 2 [0.1-30MHz]	Trig Level [0.0001-20V]	Mod [1-5]	Ampl [1-8]	Dem [0-8]	Note	Done	Report
S_1	Wheat viable	AmphaCalib	E00231	2	12	0.1	3	6	2		0	1
S_2	Wheat viable	AF6	E00231	2	12	0.1	3	6	2		1	1
S_3	Wheat viable	AF6	D00342	2	12	0.05	3	6	1		1	1
S_4	Wheat dead	AF6	D00342	2	12	0.05	3	6	1		1	1
S_5	Wheat microspore bicell / 3	AmphaCalib	D00342	2	12	0.05	3	6	1		1	1
S_6	Wheat mix	AF6	D00342	2	12	0.05	3	6	1		1	1
S_7	Wheat dead	AF6	D00342	2	12	0.05	3	6	1		0	1

Results

Id	Sample Name	Date/Time	Duration[mm:ss]	Concentration[cells/ml]	MeanFlow[ul/min]	MeasVol[ul]	Accepted	Rejected[%]
S_2	Wheat viable	09-Mai-2017 11:19:48	00:35	2'054	2402.5	1403.0	2'882	3.2
S_3	Wheat viable	09-Mai-2017 11:30:00	01:30	4'725	473.5	711.6	3'363	4.3
S_4	Wheat dead	09-Mai-2017 11:52:54	01:41	4'069	483.1	814.6	3'315	7.6
S_5	Wheat microspore bicell / 3	09-Mai-2017 11:38:26	01:43	10'809	448.2	770.4	8'328	4.1
S_6	Wheat mix	09-Mai-2017 11:55:58	02:26	16'319	480.5	1170.7	19'105	4

From Sample to Result - Advanced window



The screenshot displays the AmphaSoft 2.0 software interface, specifically the Advanced window. The interface is divided into several sections:

- Oscilloscope window:** A large central area showing a plot of Amplitude [V] versus Length [ms]. The y-axis ranges from -3 to 3, and the x-axis ranges from 0 to 10. The plot area is currently empty.
- Gain settings:** A panel with three sliders for Modulation, Amplification, and Demodulation. The Amplification slider is currently set to 6.
- Trigger Algorithm:** A panel with controls for Level [V] (0.10000), Source (x), and Direction (+).
- Frame size:** A panel with controls for Length [ms] (10) and Amplitude [V] (3.00), along with checkboxes for x, y, and trig.
- Pump control:** A panel with an On checkbox, Speed [rpm] (80), and Direction (Counter Clockwise).
- Valve control:** A panel with Left and Right checkboxes.
- Process standards:** A panel with checkboxes for Initial Rinsing, Chip Detection, Load Sample, and Flush.
- Pulse control:** A panel with an On checkbox, Amplitude [snr] (0.001), Duration [ms] (0.10), and Direction (Positive).
- Log window:** A panel at the bottom showing a log of system events, including measurement loading, recorder status, and oscilloscope version information.
- Functions:** A vertical sidebar on the right with radio buttons for Oscilloscope, Triggering, AutoGain, and Chip Test, and a Start button.

Maintenance – The best way to prevent errors



Maintenance – Instrument Shutdown



- Cleaning of the fluidics with AmphaClean solution
- After each series of measurements, at the end of the day
- Can be performed with
 - Measurement Chip
 - Cleaning Chip
- Procedure
 - Configure correct chip
 - Open the *Basic* tab and check *Cleaning*
 - Press *Start Rinsing* and follow the instructions
 - Place a beaker below the sample aspiration tube > Confirm
 - Check bottles > Confirm
 - Place a sample of AmphaClean > Confirm
 - Remove the AmphaClean tube and place a beaker > Confirm
- At the end, empty waste bottle, click *Disconnect* in the *Admin* tab and switch off the instrument

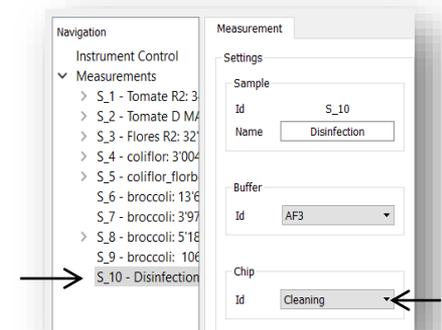


References *Ampha Z32 User Guide, Maintenance*



Maintenance – Instrument Disinfection

- Disinfecting the instrument fluidics with 70 % ethanol
- On a regular basis, e.g. once per month or when the instrument will be idle for a longer period
- Can be performed with the Cleaning Chip only (!), as measurement chips are not compatible with organic solvents
- Procedure
 - Create a new measurement in the *Worklist*
 - Open the newly created measurement in the left panel, e.g. S_10
 - Configure *Cleaning* chip in the *Measurement* tab
 - Open the *Basic* tab and check *Disinfection*
 - Press *Start Rinsing* and follow the instructions
 - Place a beaker > Confirm
 - Check bottles > Confirm
 - Place a sample of 70 % ethanol > Confirm
 - Remove the ethanol tube and place a beaker > Confirm



References *Ampha Z32 User Guide, Maintenance*

Maintenance - Storage



Measurement Chips

- Store in a dark and dry environment
- Regular Chip Tests to ensure measurement validity



Buffers

- Store in the fridge at 4°C
- Check the quality before using
 - Clean solution, no solids
 - No fungal growth
- Use aliquots for measurements, e.g. in 50 ml Falcon tubes
- Use buffers only after equilibrating to room temperature



References Ampha Z32 User Guide, Setting up the Ampha Z32 Impedance Flow Cytometer



Maintenance – Routines

- Daily
 - Cleaning with AmphaClean
 - Measurement Chip or Wash Chip
 - *Basic Tab > Cleaning > Start Rinsing*
- Weekly
 - Cleaning with 70 % ethanol
 - Wash Chip only
 - *Basic Tab > Disinfection > Start Rinsing*
- Monthly or if requested
 - Check impedance of chips
 - *Tools > Chip Test > Select Chip > Plug AmphaCalib sample > Start*
- Quarterly
 - Replace tubing set
- Anually
 - Service contracts

Maintenance – Annual Service Contracts



Support and Maintenance activities		Support and Maintenance Plan	
		Standard	Profess.
A	Support for the use of the System	x	x
	Response Time for Support requests	2 days	24 hrs
	Telephone hotline for critical issues		x
B	<p>One on-site visit per year with operational check and Maintenance of the Instrument and Software, including:</p> <ul style="list-style-type: none"> • Installation of Software and Firmware Updates • Explanation and training of new functions • Exchange of fluidic set (material included) • Clean valves • Clean chip head and driver board • Check correct valve functions • Check of pump, grease if necessary • Check of all interior tubes • Check pressure spring (correct pressure and smoothness of travel) • Check correct fit for chip interface lid • Check correct positioning for plexiglas cover • Check chip contact pins • Check of flow sensor • Check of electronics (boards, LEDs etc.) • Check USB port and yellow cable • Valuation of all AlphaChips 	x	x
B'	Replacement of all interior tubes (material included)	x	x
C	Materials for quarterly maintenance included	x	x
D	Questions and answers workshop	x	x
E	Expert training	x	x
F	All service materials and work time included		x
G	Free consumables (buffers) and free replacement of out-of-spec chips		x
H	Free replacement Instrument (express shipping)		x
I	Extended Warranty during term of Agreement		x
J	Free Amphacademy participations	2	any



Maintenance – Annual Service Contracts

- Price for Professional Contract – 5'400 CHF
- Price for Standard Contract – 3'200 CHF
- Majority of customers decide for a maintenance plan
 - ~ 60% Professional; 40% Standard

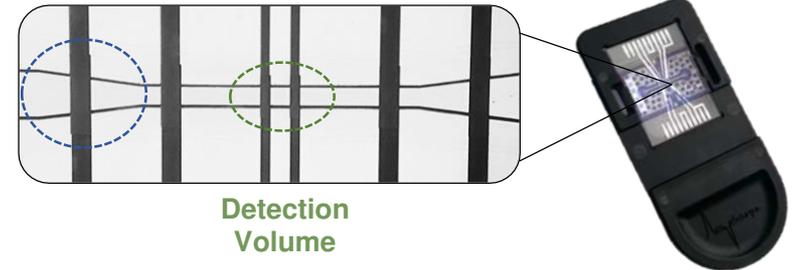
Tips and Tricks



Chip Clogging

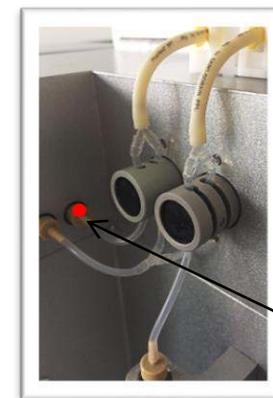
- Stop the measurement | Click TWICE!
- Remove the chip
- Unclog the chip using the wash station
- Re-insert the chip
- Re-attach the tubing if disconnected
- Perform a rinsing

Narrowing

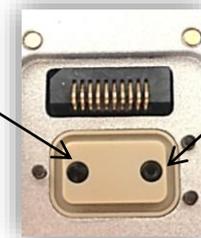


Cleaning the chip block

- Syringe with soft tip
- Left and right port of chip block
 - Remove silicone tubing for left port
- Carefully, do not apply to much pressure



Left port



Right port

Remove this tube to clean the left port

References *Ampha Z32 User Guide, Maintenance*



Valves

- Test valves before measuring
 - Go to the *Advanced* tab
 - In the *Valve* section, click the two boxes for the left and right valves
- In case the valves are blocked/ moving slowly: rinse them with water according to the instructions in the user manual
- In case valves are not moving at all: Check the cables in the back of the instrument

Water bottle

- Exchange deionized water on a regular basis

Waste bottle

- Empty and clean bottle after every use

References *Ampha Z32 User Guide, Tips & Tricks*
 Ampha Z32 User Guide, Maintenance



Field Use

- Please use only Amphasys batteries
 - Check the battery status using the Check button
 - Recharge when only 1 LED is on
 - When the battery is fully charged, the 4 LEDs turn off
- ! Do not use power generators to power the instrument, as can produce voltage spikes !
- Work in an environment protected from high temperatures, direct sunlight, dust, rain, high humidity...
- Pack and transport the instrument carefully

References Amphasis battery pack user guide



Further information

- Pollen list with buffer, filter and chip recommendations
www.amphasys.com/download
- Tutorial movies
www.amphasys.com/tutorials
- Ampha Z32 User Guide
www.amphasys.com/download or in AmphaSoft under *Help*
- AmphaSoft 2.0 User Guide
www.amphasys.com/download or in AmphaSoft under *Help*
- Online help, Tips and Tricks
www.amphasys.com/download
- News
www.amphasys.com/news and www.linkedin.com/company/amphasys
- Contact us!

Amphasys Support - Who you gonna call?



- If all your efforts cannot solve the issue
- support@amphasys.com
- Ensure you have all the data (WS, brief history of the error, pictures, your efforts so far) ready **before** approaching us [**We will ask for that!**]
- Having everything ready at your first approach saves a lot of time

Your Contacts



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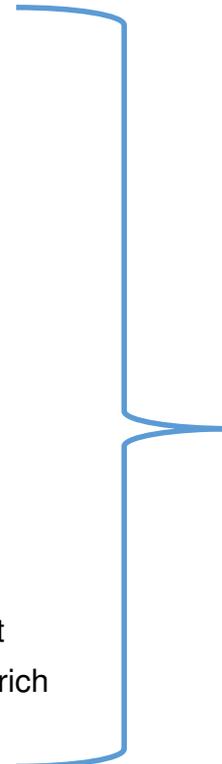
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