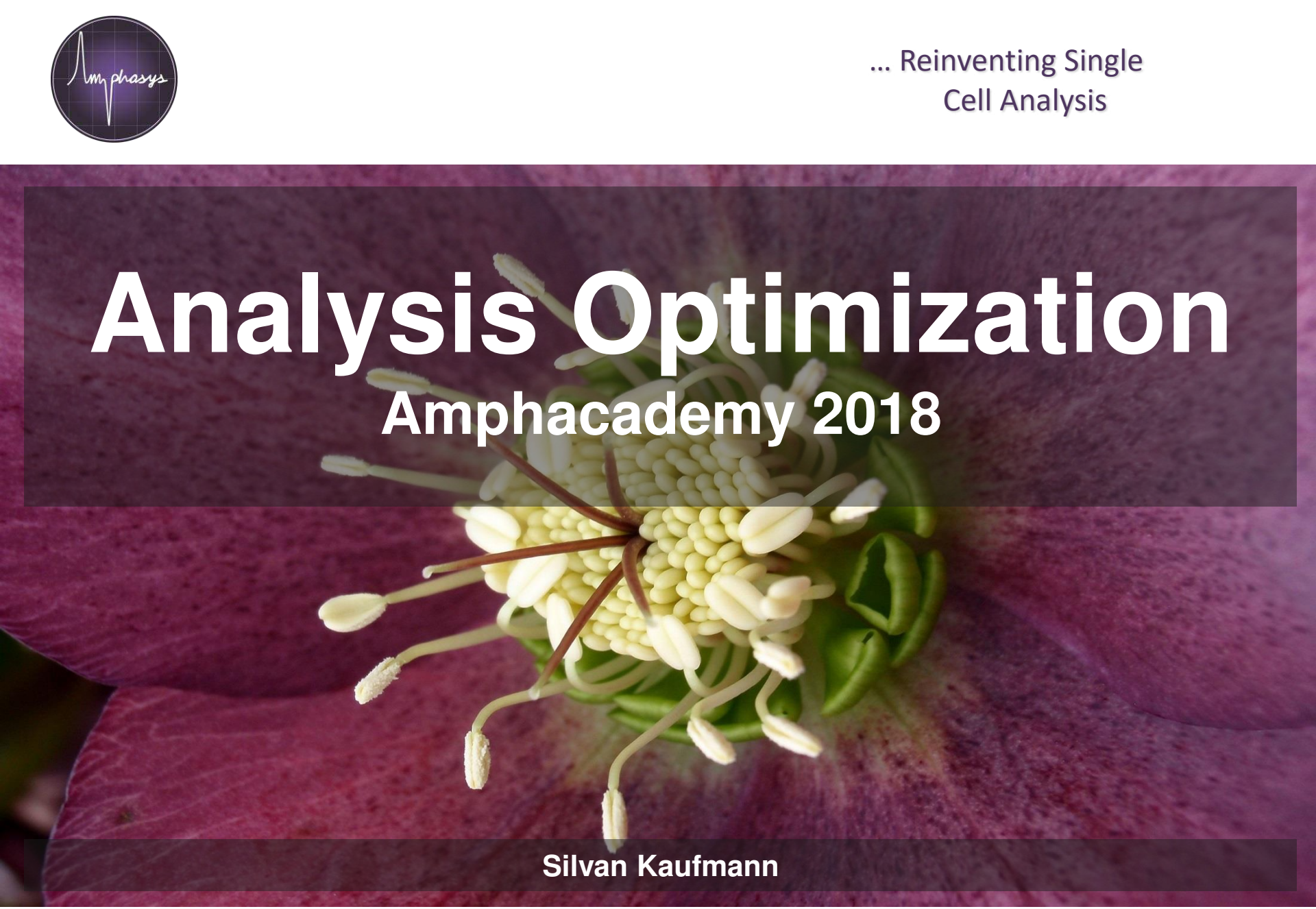

… Reinventing Single Cell Analysis

Analysis Optimization

Amphacademy 2018

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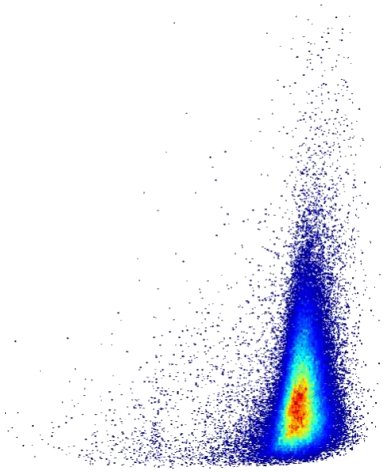
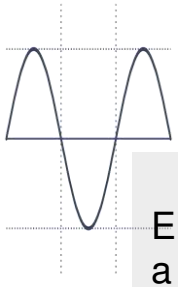
Contents

- Introduction
- Gating strategies
- Advanced gate statistics
- Exporting of data
- Reporting



Introduction

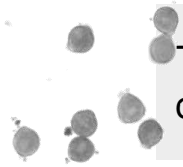
Impedance Flow Cytometry (IFC)



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

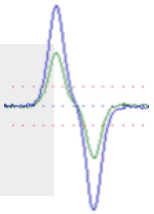
The cells are suspended in a conductive **Fluid**

Impedance Flow Cytometry



The technology is used to characterize **Cells**

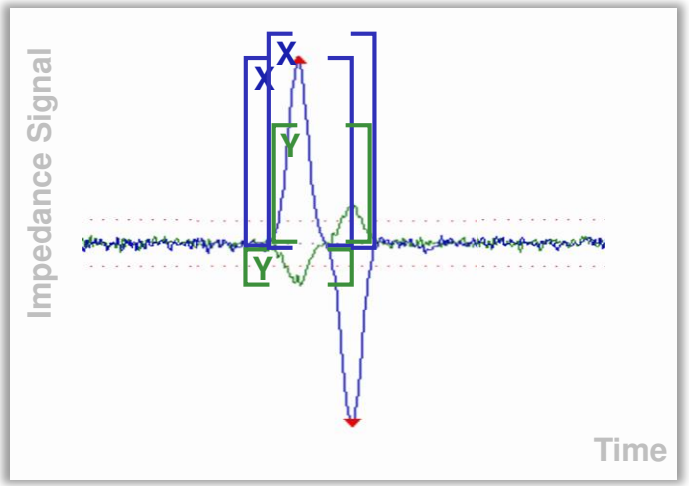
Electrical properties of cells are **Measured**



How we measure cells…

Viable Cell

Dead Cell



Real Part (X)

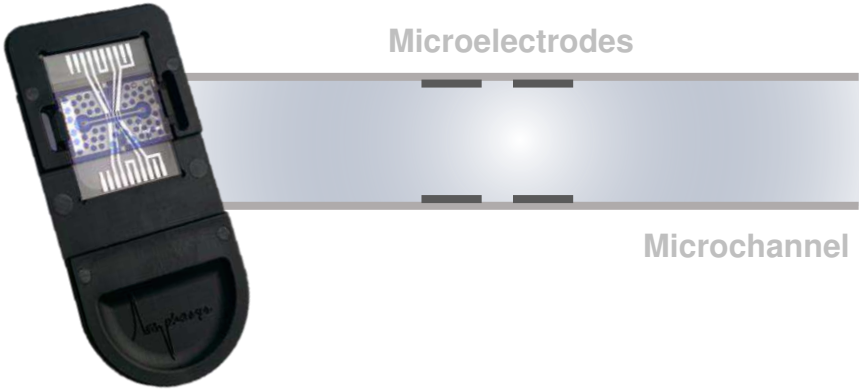
Imaginary Part (Y)

Peak Positions

Triggering Levels

Sample

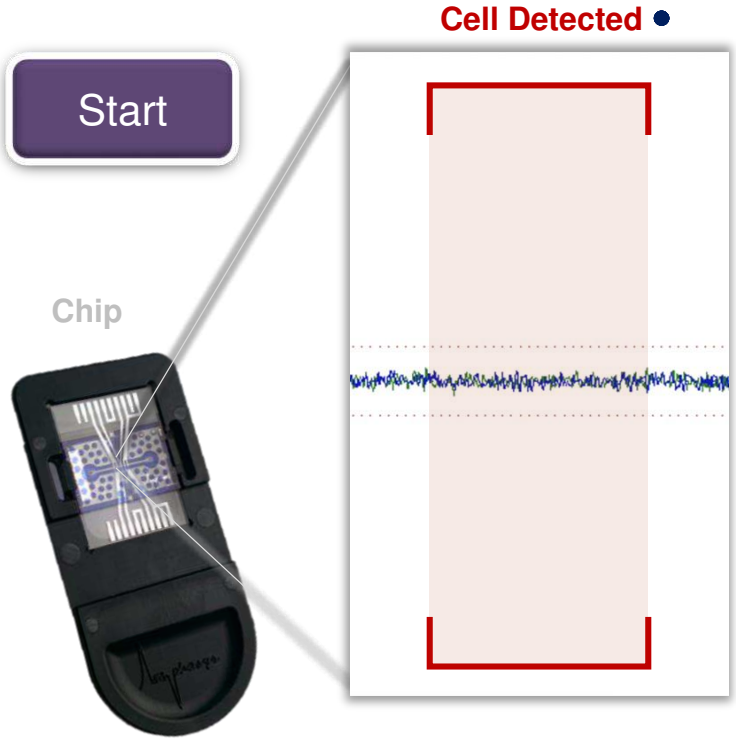
Chip

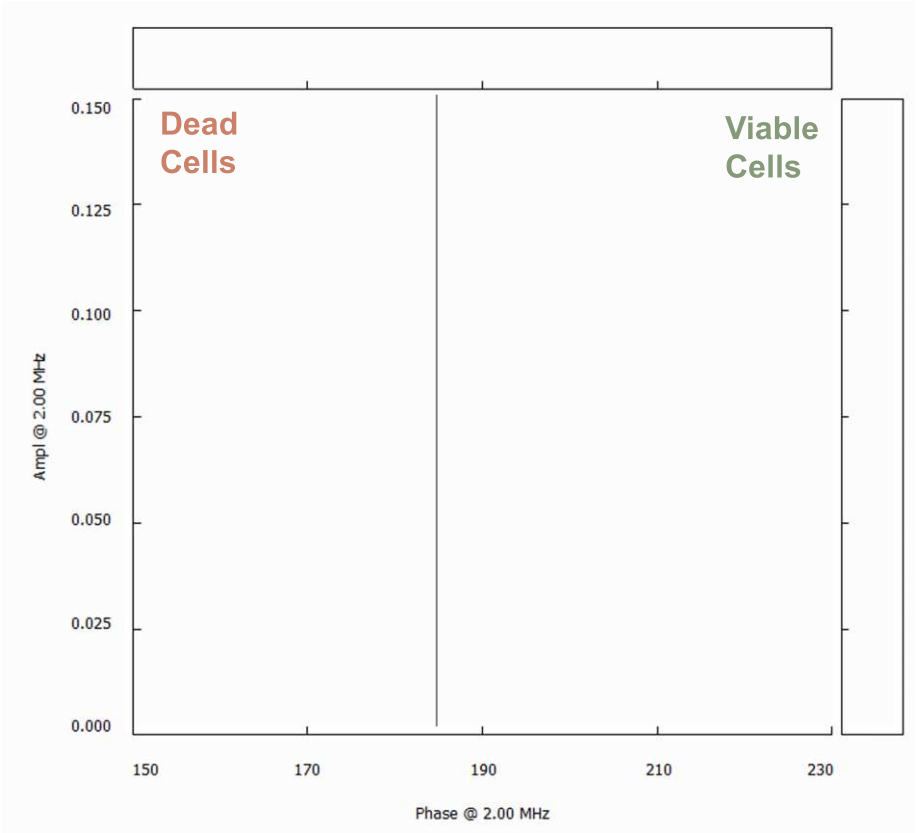


How we measure cells…

Impedance Signals

Phase – Amplitude Scatterplot





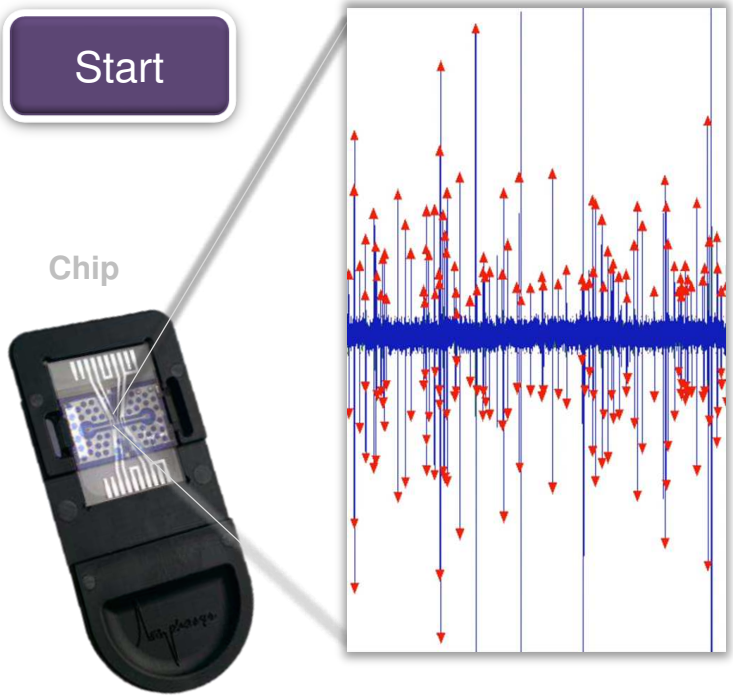
…lots of cells

Impedance Signals

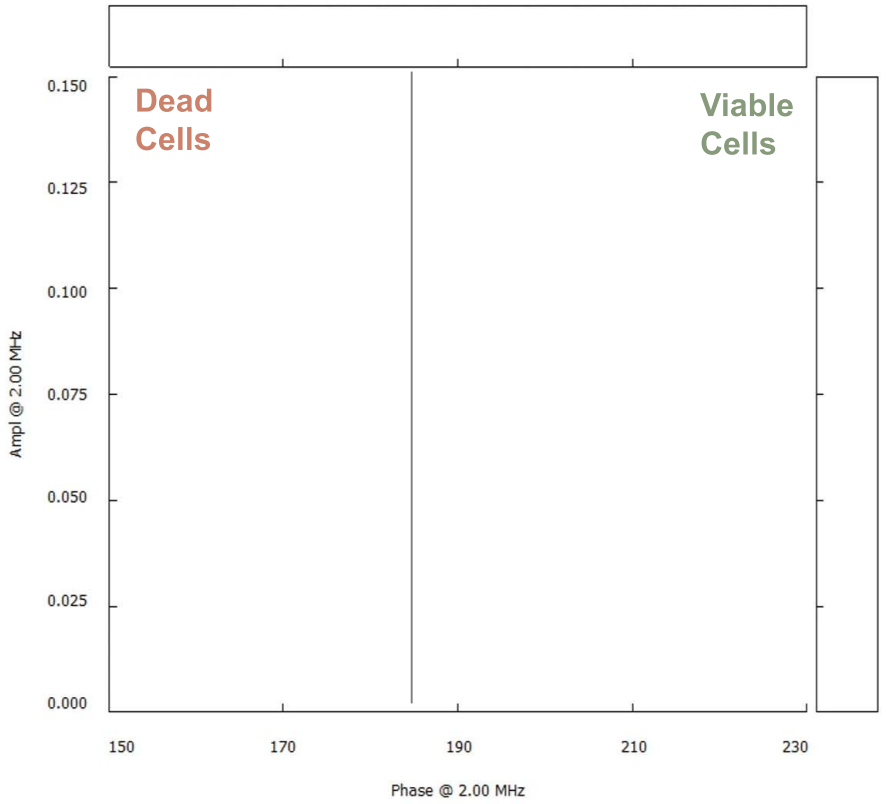
Real-time cell analysis

Start

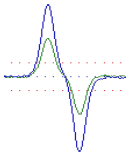
Chip



Phase – Amplitude Scatterplot



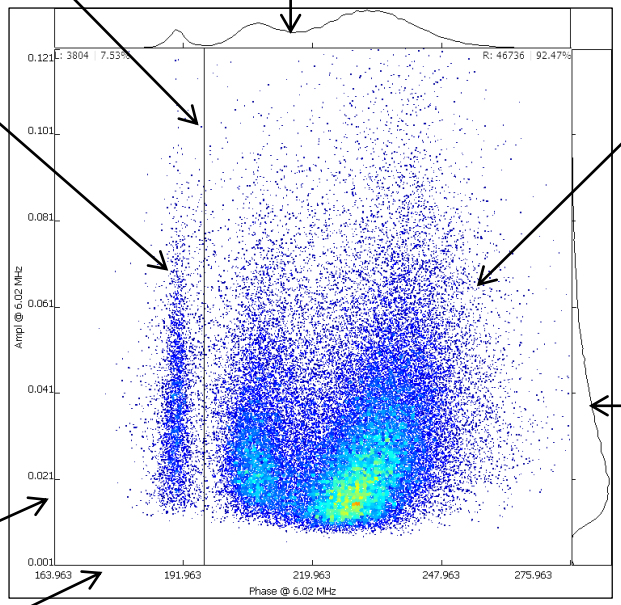
The AmphaSoft Scatterplots



Each measured particle corresponds to one dot in the density scatterplot

Vertical Gate

Phase Histogram



Density Scatterplot revealing electrically different cell populations

Amplitude Histogram

Amplitude Axis

Phase Axis

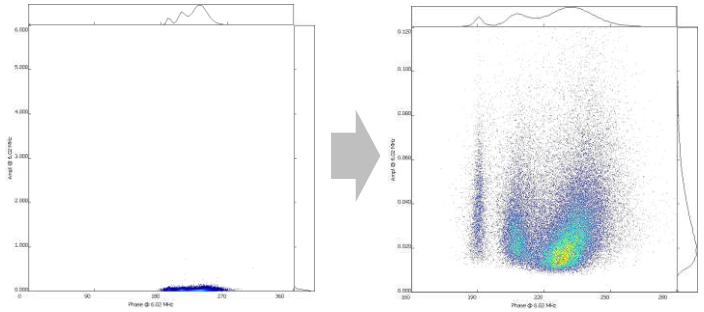
Dead cells appear at low phase angles

Viable cells appear at higher phase angles

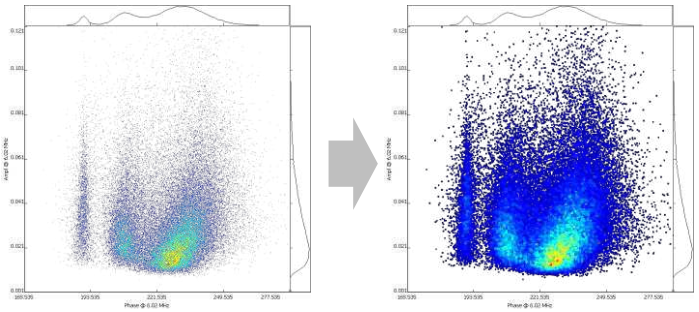
Data Analysis

Visualization

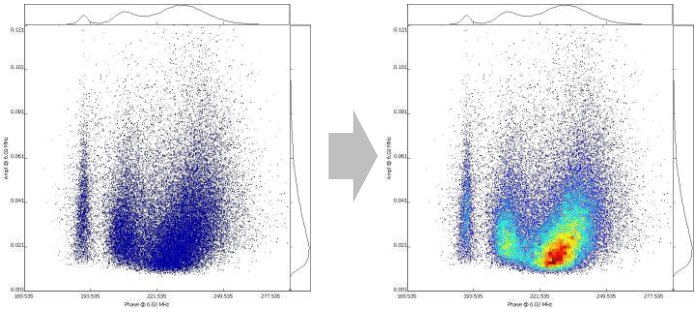
- Zooming the data properly
  - Autozoom feature (new)
  - Mouse wheel
  - Axis limit settings



- Changing the dot size
  - Press *Ctrl* and move the mouse wheel in the plot



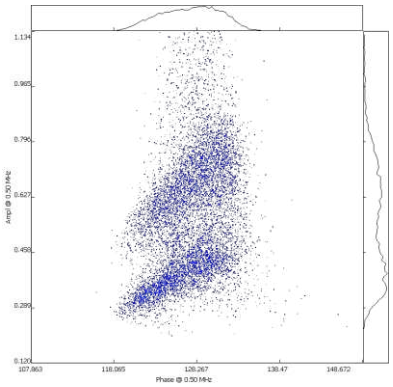
- Changing the density plot coloring
  - Press *Alt* and move the mouse wheel in the plot



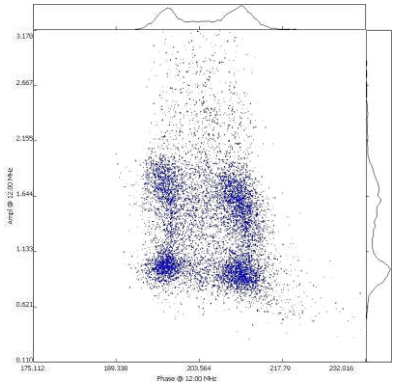
Frequency and gating strategy

- Data analysis at one frequency is sufficient
  - i.e. gates only need to be placed in one plot
  - Just take the plot that is more suitable
    - Typically plots at 12 MHz show a better resolution between subpopulations and have less pronounced phase and amplitude drifts
- The gating strategy depends on the plot and the question you want to answer.
  - E.g. What is the cell viability
    - of mature cells?
    - of all cells?
  - What is the percentage of sterile or immature cells?
  - What is the concentration of pollen cells in the sample?
  - Ploidy?

Sunflower at 2 MHz



Sunflower at 12 MHz



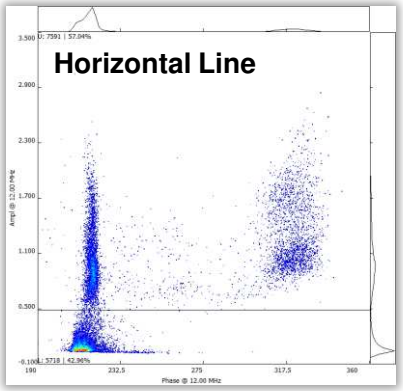
Gating

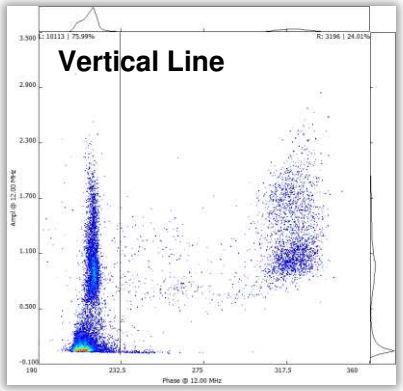
- Horizontal line gate
- Vertical line gate
- Cross gate
- Polygon gate
  - Hide cells
  - Advanced statistics

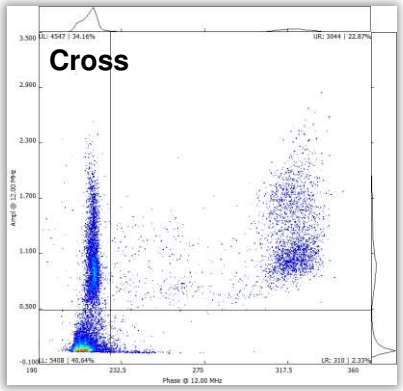
What is the meaning of the gate labels?

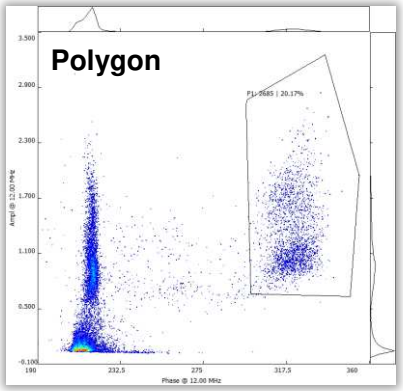
- R: Name of the gate

- 31438: Number of points inside the gate
- 91.18 %: Percentage of points in the gate, with respect to all points in the plot







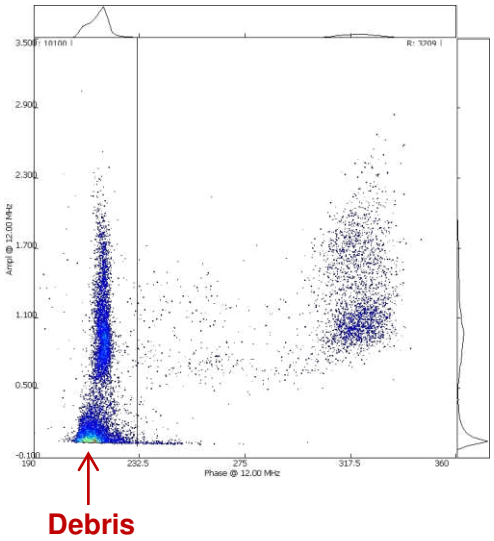


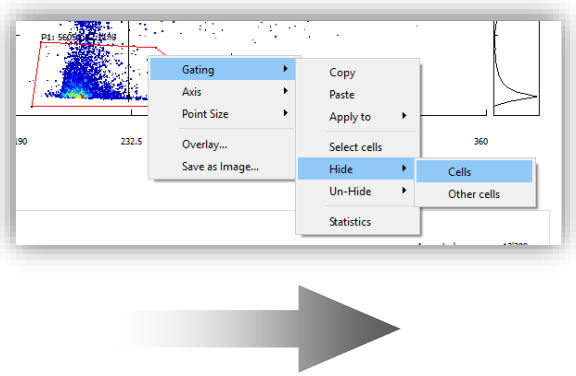
Hide Cells

- *Hide Cells* to exclude debris from the analysis
- Hide *polygon gate* content or everything around the polygon
- Apply *Hide Cells* gate to other measurements as usual

Viability before correction

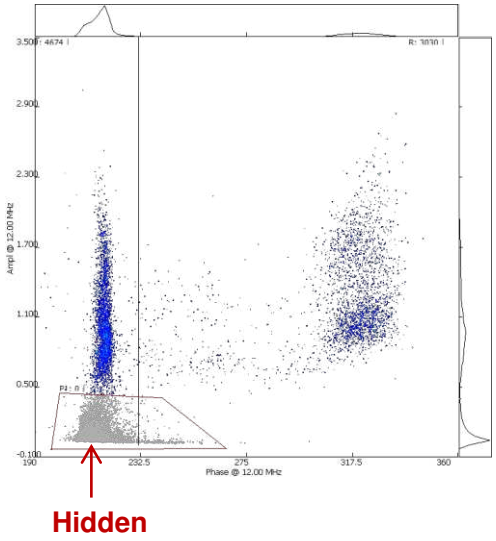
24.1 %





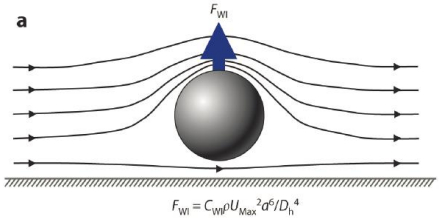
Viability after correction

39.4 %

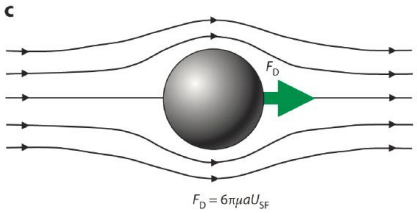


Inertial Focusing – Data Interpretation

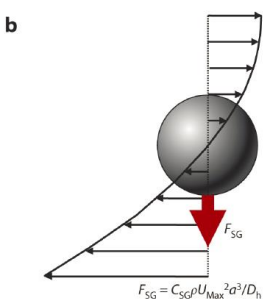
Wall-induced lift force



Viscous drag force



Shear-induced lift force



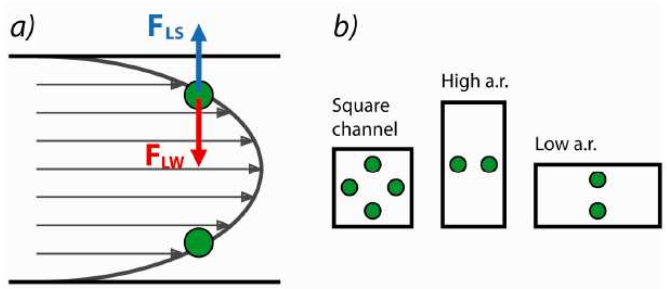
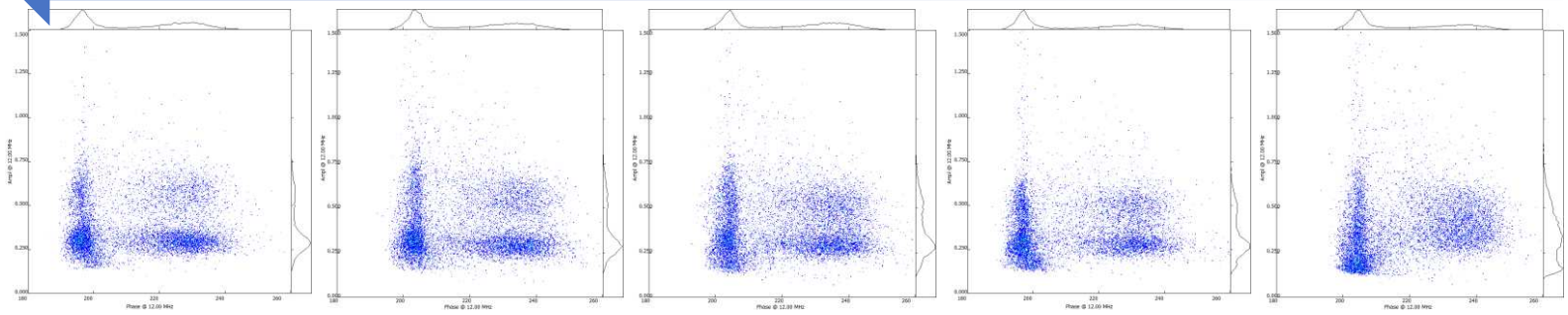


Figure 1: Particle inertial focusing in flow through straight channels. (a) The shear induced lift force (F_{LS}) and a wall induced lift force (F_{LW}) acting on a particle flowing in a microchannel. (b) Illustration of the cross-sectional equilibrium positions of particles flowing through different channel geometries.

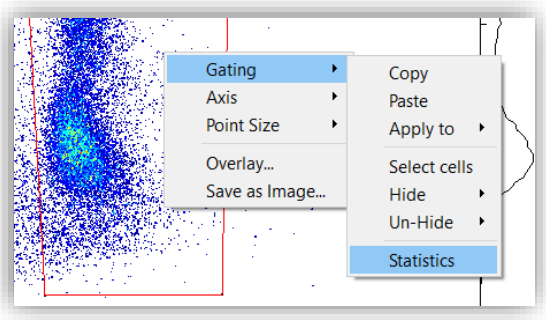
160 rpm **120 rpm** **80 rpm** **40 rpm** **10 rpm**

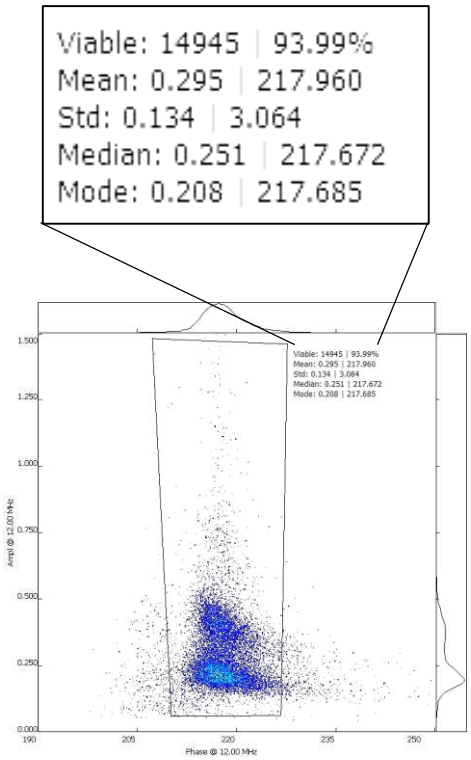
Increasing Flow Rate



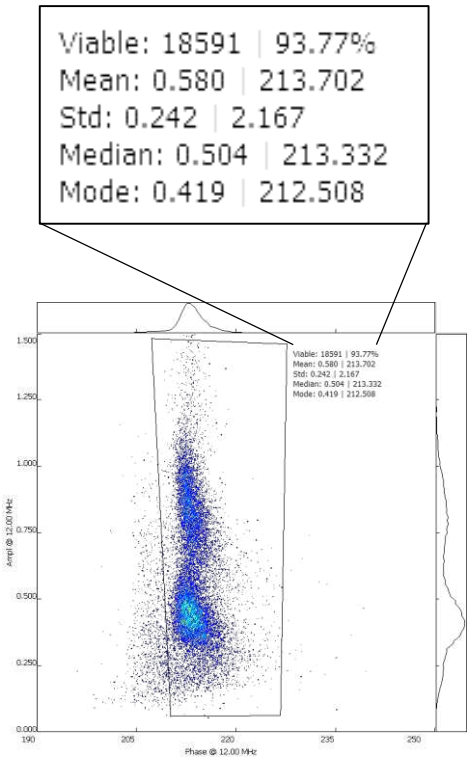
Advanced Gate Statistics

- *Mean*, *Median*, *Standard Deviation* and *Mode* of amplitudes and phases of all points in a *polygon gate*





Cyclamen 2n

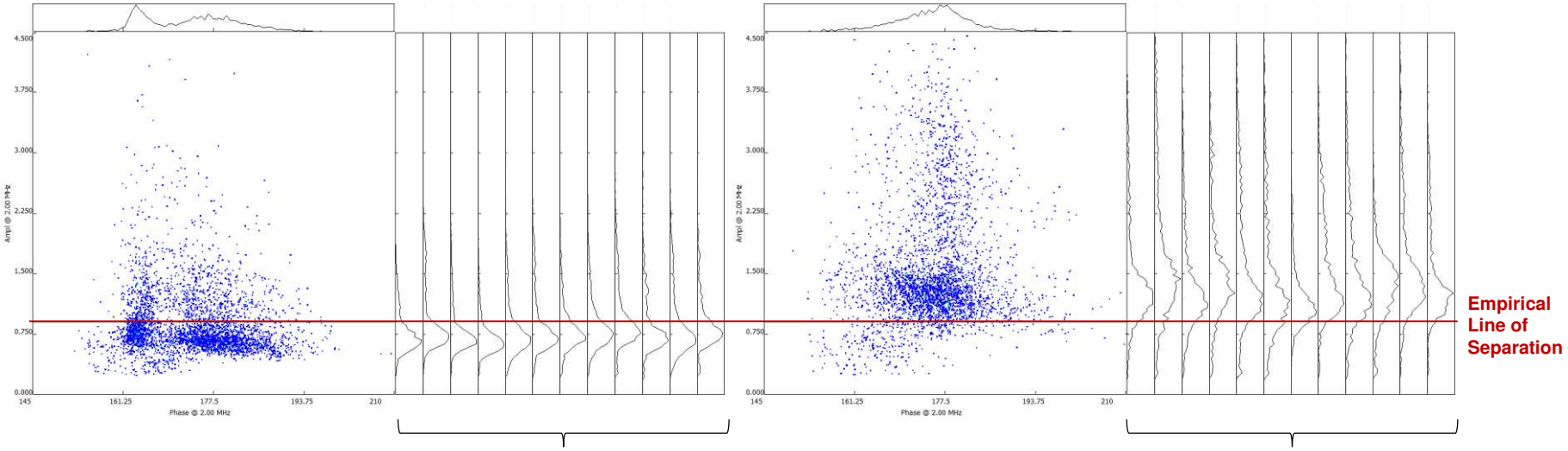


Cyclamen 4n

Pollen ploidy based on pollen size differences

2n Genotypes

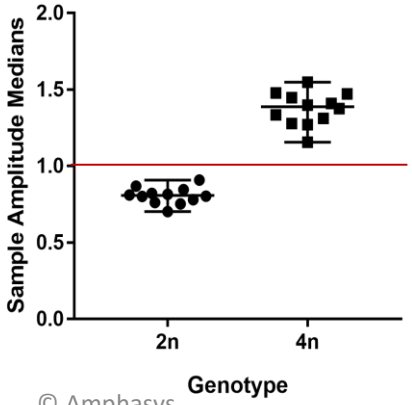
4n Genotypes



Y-Axis projections of different 2n genotypes

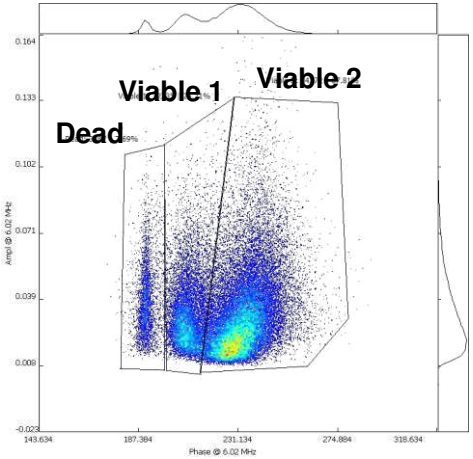
Y-Axis projections of different 4n genotypes

Median Amplitudes

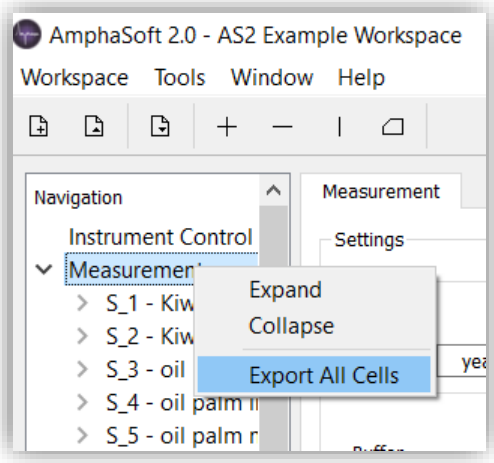


Data Export

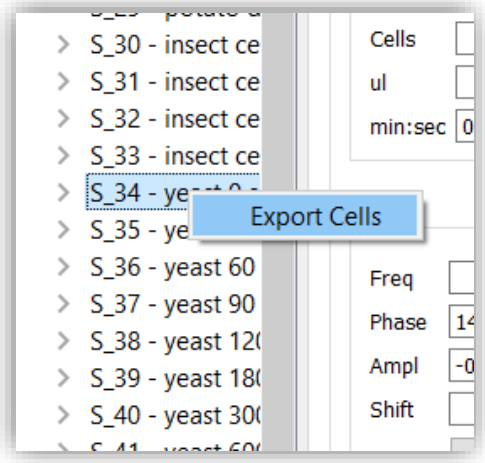
- For advanced data analysis with custom algorithms
- Phase, amplitude and corresponding gate of each particle in *.csv* format
- Export of all data
  - Right-click on Measurements > *Export All Cells*
- Export of single-measurement data
  - Right-click on measurement > *Export Cells*



Export of all data



Export of single measurement data



Phase / Amplitude / Gate Data

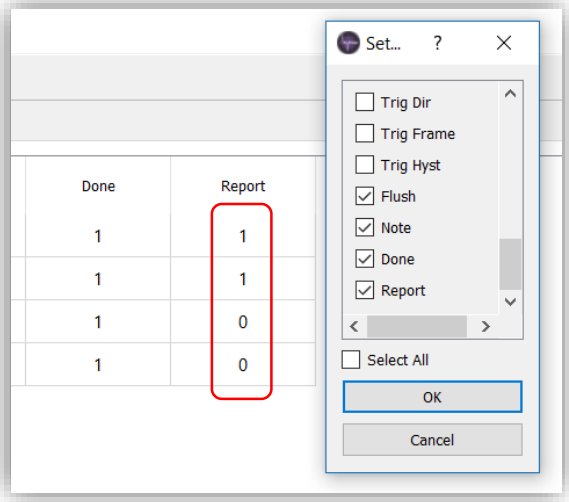
	A	B	C
1	247.485	0.059732	Viable 2
2	235.866	0.078129	Viable 2
3	221.102	0.07715	Viable 1
4	210.053	0.031917	Viable 1
5	206.661	0.024568	Viable 1
6	238.14	0.053583	Viable 2
7	189.229	0.071557	Dead
8	231.657	0.022509	Viable 2
9	243.51	0.030326	Viable 2
10	223.015	0.049962	Viable 2
11	253.639	0.050914	Viable 2

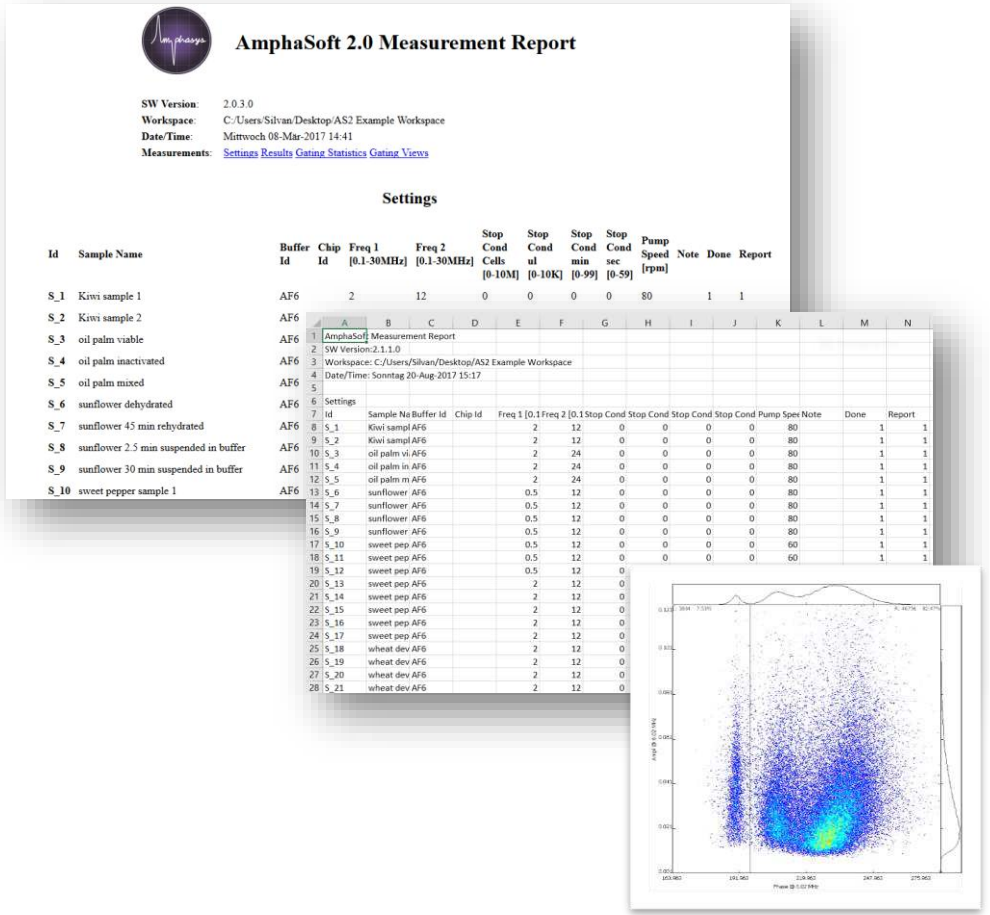
Reporting and Plot Export

- *.csv* report
- *.html* report

Select which measurements you want in the report!

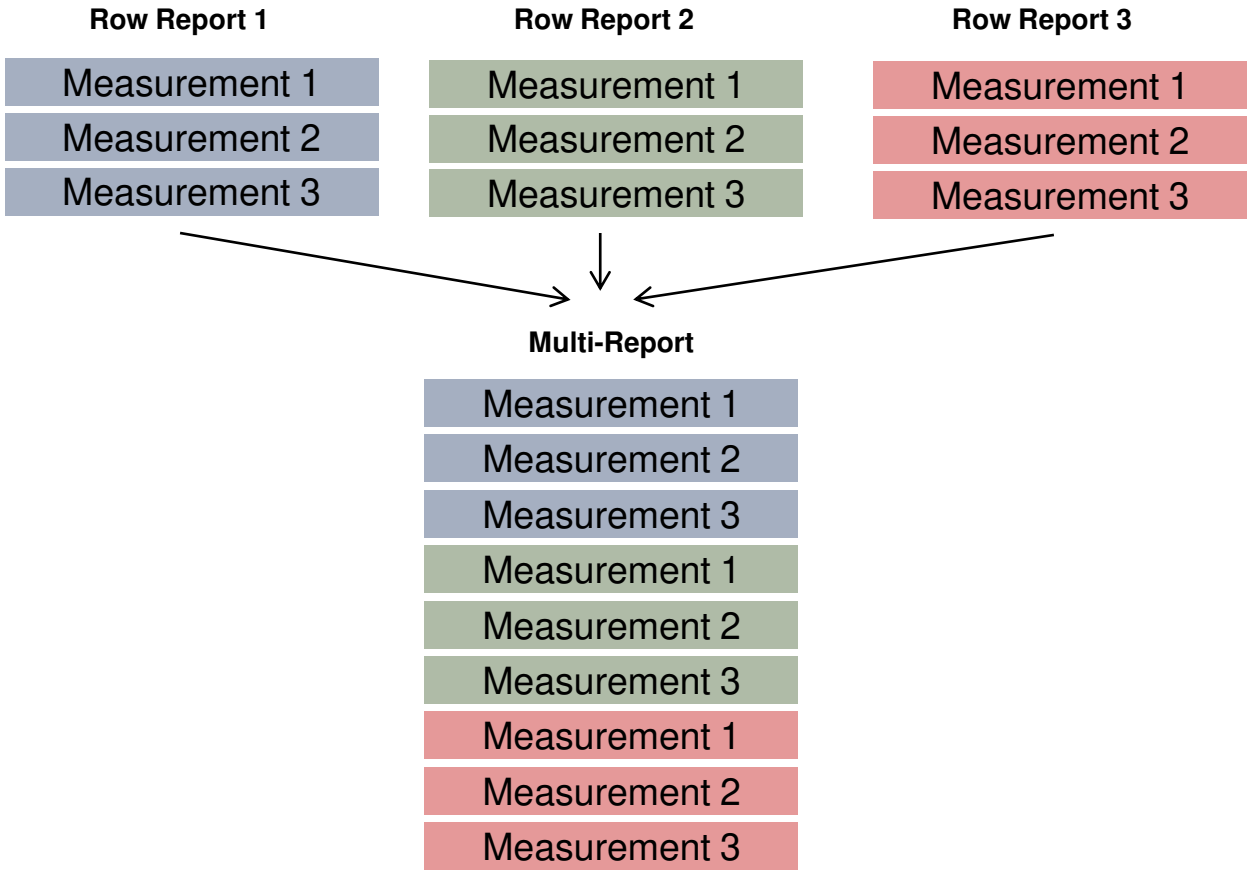
- Plot export





New feature: Merging of reports

- New report type called «row report»: All reported settings and results or one measurement are reported in one single row.
- Workspaces can be merged



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