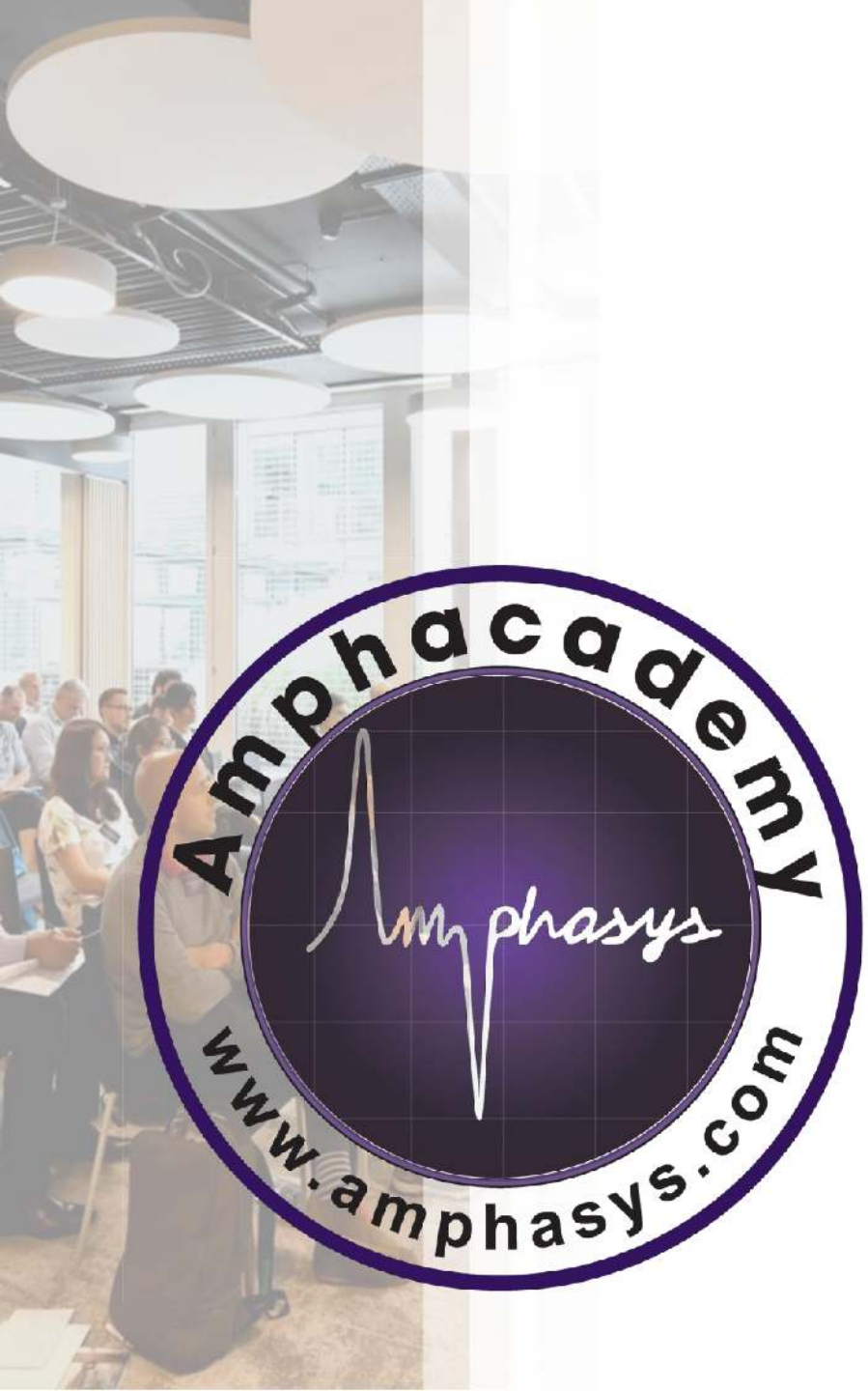


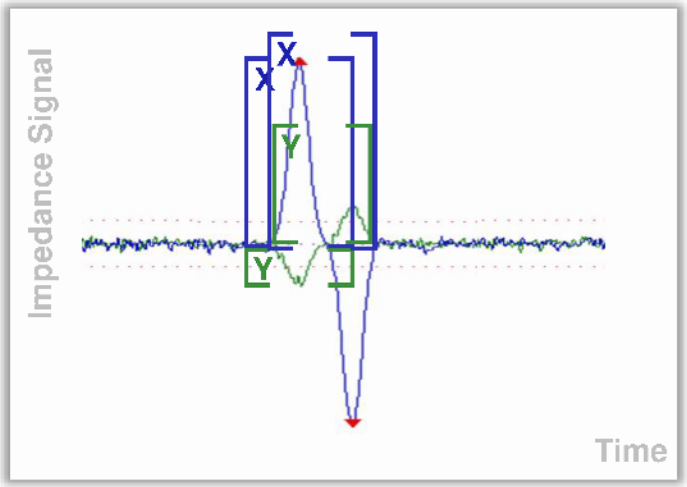
Breakout Session 4

Data Analysis

How we measure cells…

Viable Cell

Dead Cell



Real Part (X)

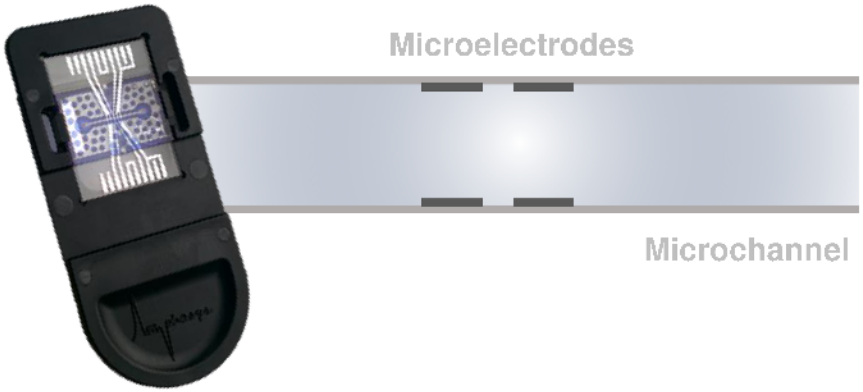
Imaginary Part (Y)

Peak Positions

Triggering Levels

Sample

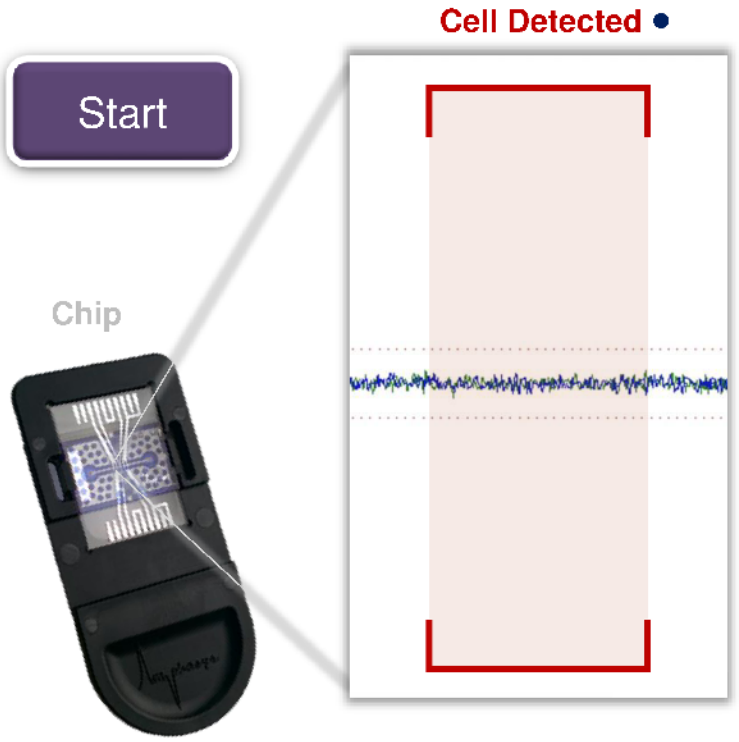
Chip

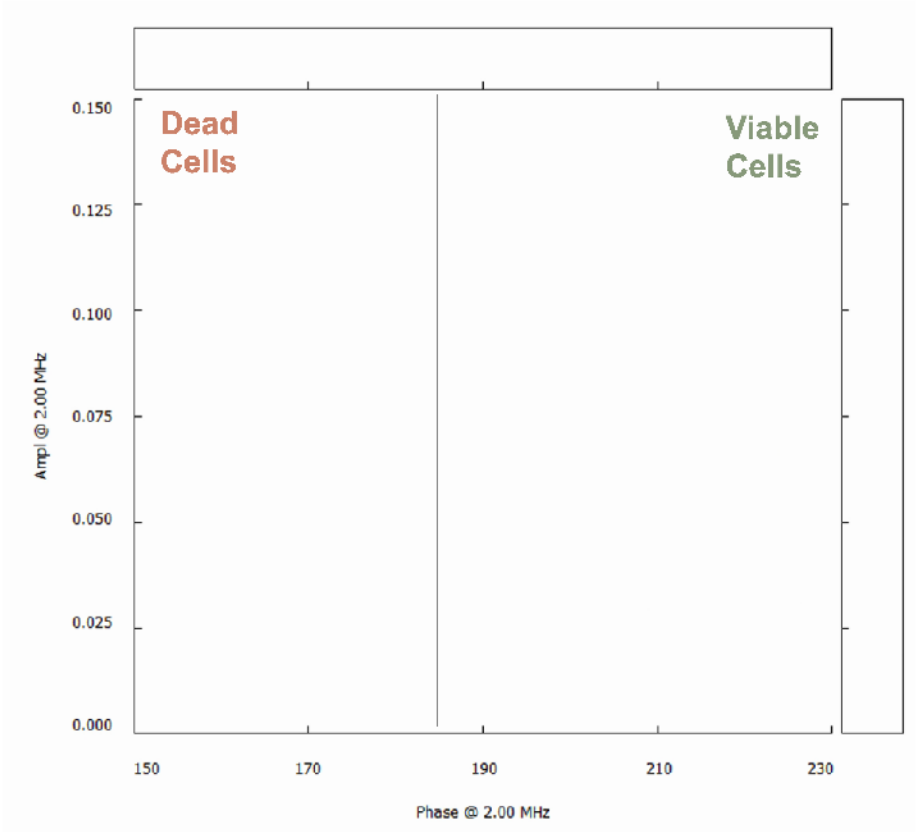


How we measure cells…

Impedance Signals

Phase – Amplitude Scatterplot





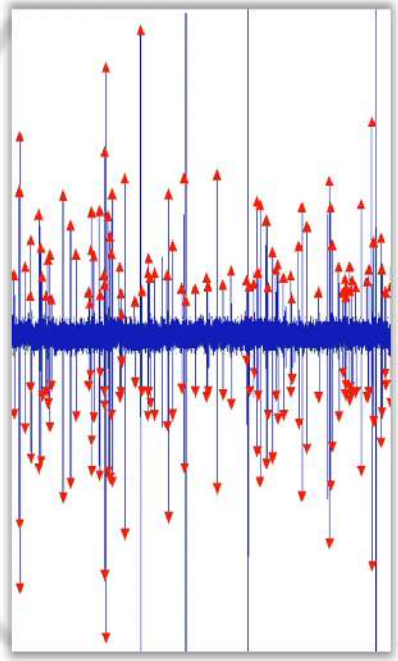
…lots of cells

Impedance Signals

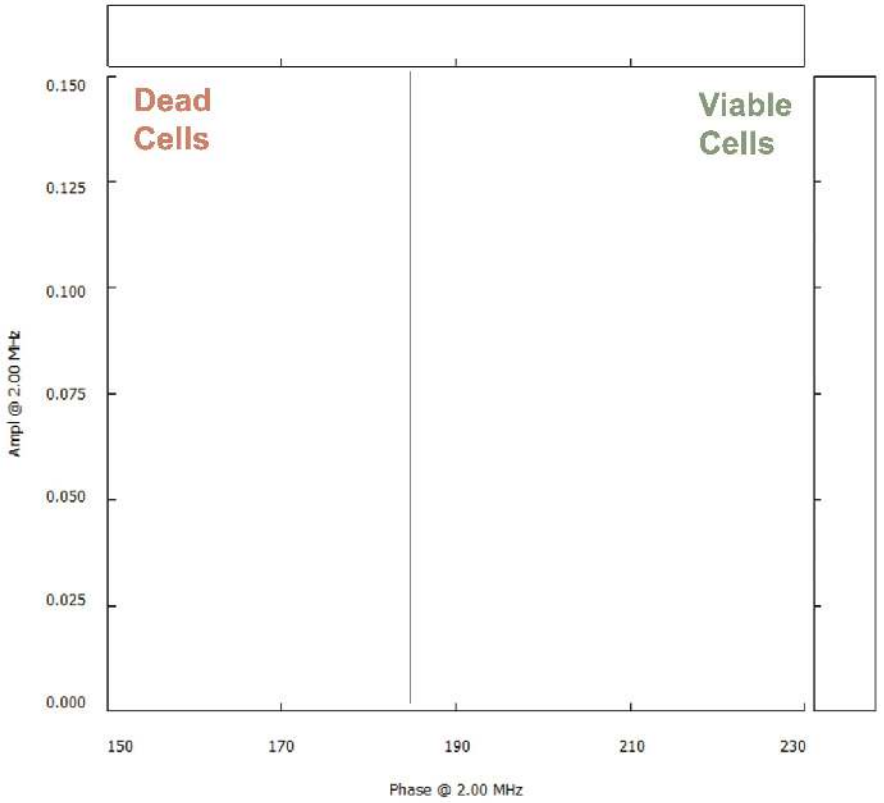
Real-time cell analysis

Start

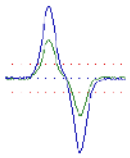
Chip



Phase – Amplitude Scatterplot



AmphaSoft Scatterplots



Each measured particle corresponds to one dot in the scatterplot

Vertical Gate

Phase Density

Density Scatterplot revealing electrically different cell populations

Amplitude Density

Amplitude Axis

Phase Axis

Impedance Amplitude

Impedance Phase

Dead cells appear at low phase angles

Viable cells appear at higher phase angles

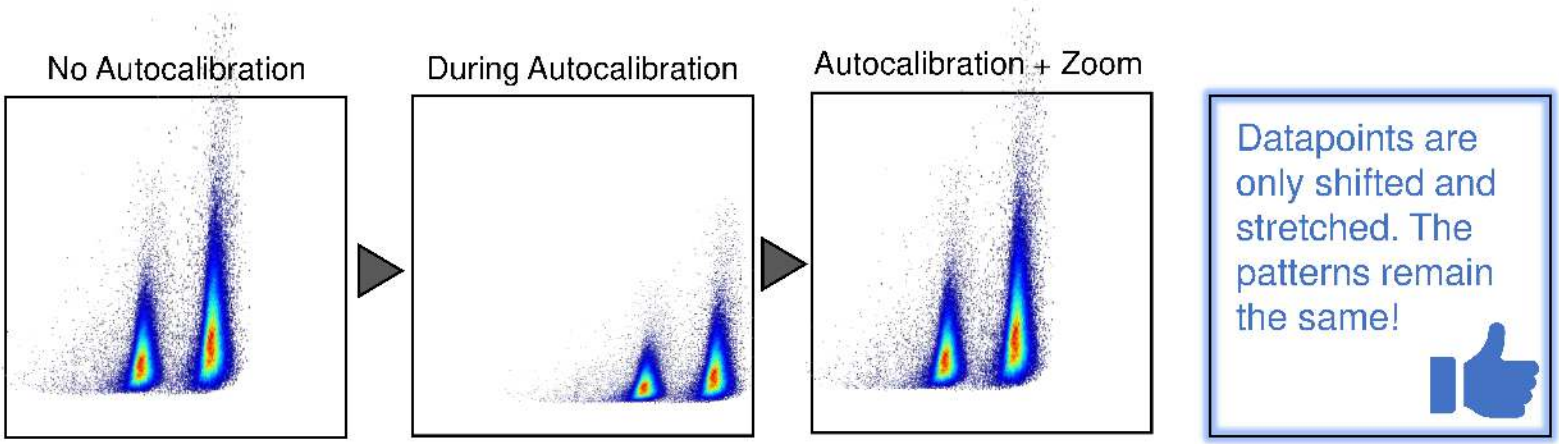
Data Analysis Features

What is new in AmphaSoft 2.1.6?

AmphaSoft 2.1.5 to AmphaSoft 2.1.6

Autocalibration

- Offset correction: Chip / Instrument / Temperature
- Fully automated
- Data analysis unchanged

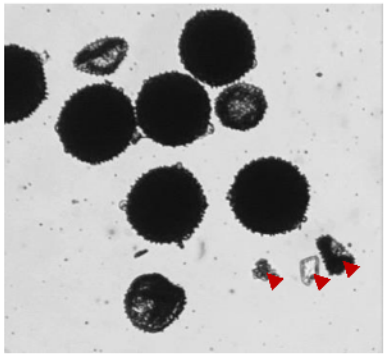


Data Patterns

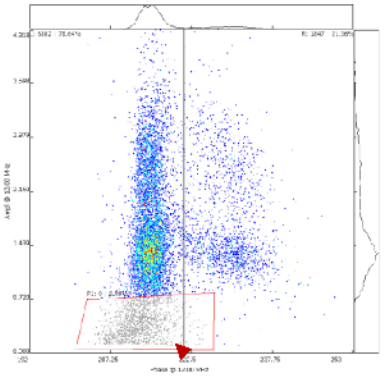
Debris

Patterns

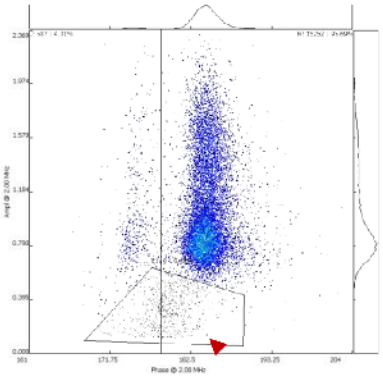
Debris



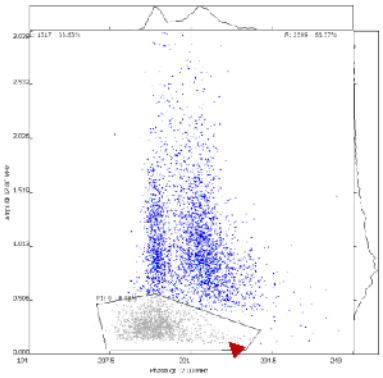
Sweet Pepper



Corn



Broccoli

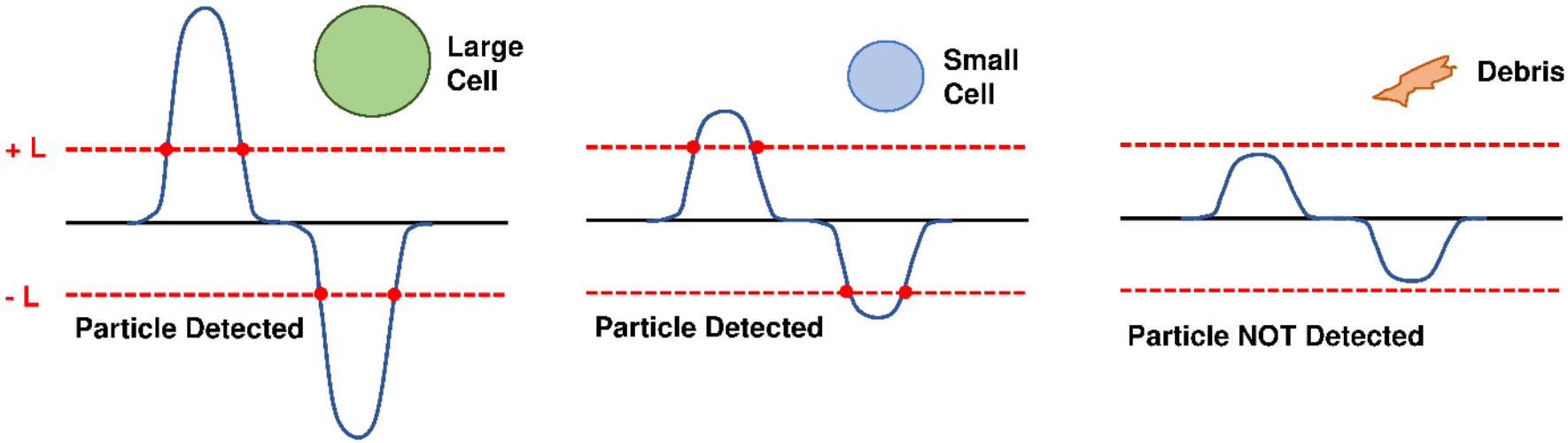


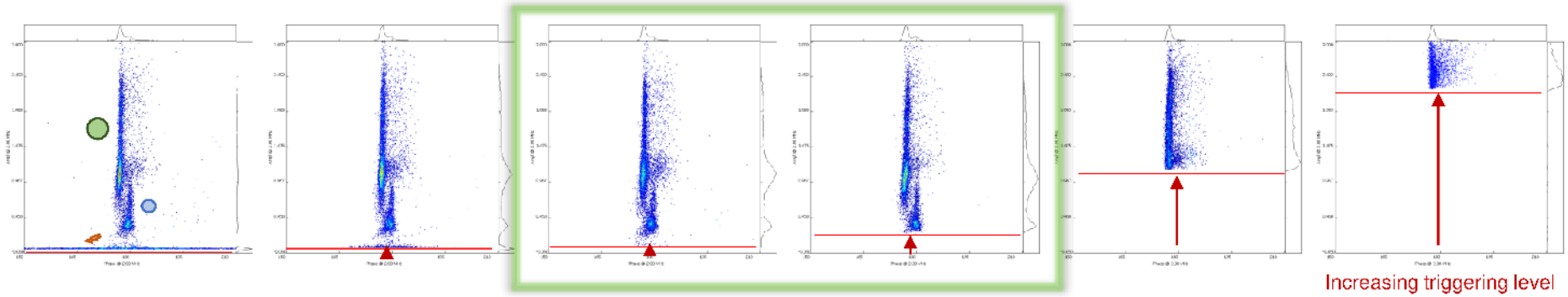
How to deal with debris

1) Sample preparation: Nondestructive methods, i.e. without using the pellet mixer
2) Data analysis: *Hide cells* feature
3) Instrument configuration: Adjusting the triggering level

Excluding debris by adjusting the level

- The triggering level is a threshold to exclude particles with a small volume

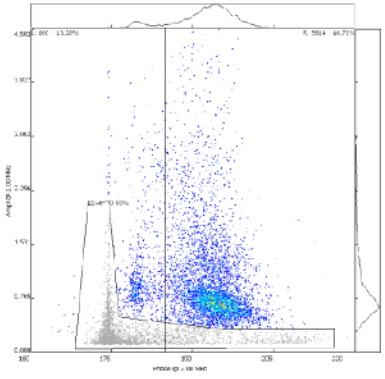




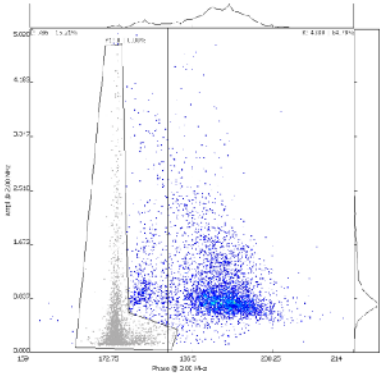
Air bubbles

- Observation

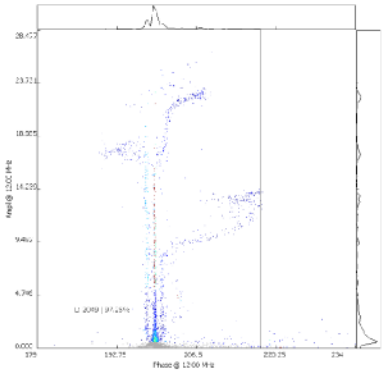
Watermelon



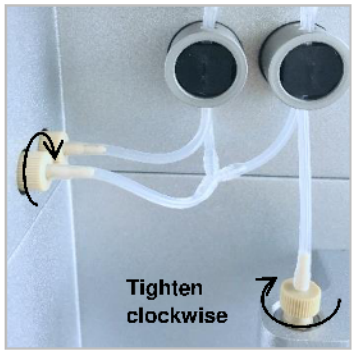
Cucumber



Foam

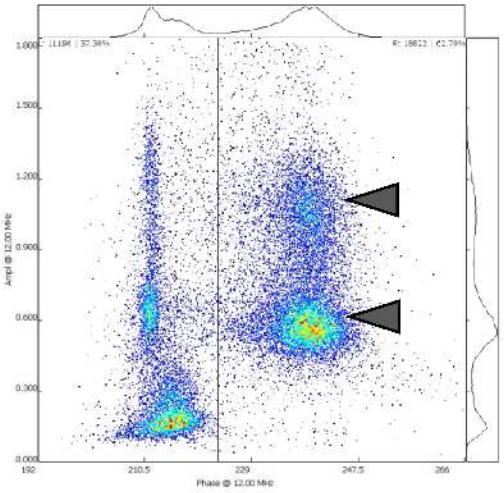


Tightening barb adapters



- Pattern comes from air bubbles. Air bubbles are electrically not conductive, and therefore they give us an impedance signal as well.
- Resolution
  - Hide air bubble population using the *Hide Cells* feature
  - Adjust sample preparation (do not shake sample excessively before measuring)
  - Make sure fluidic system is not leaky

Inertial Focusing – Data Interpretation



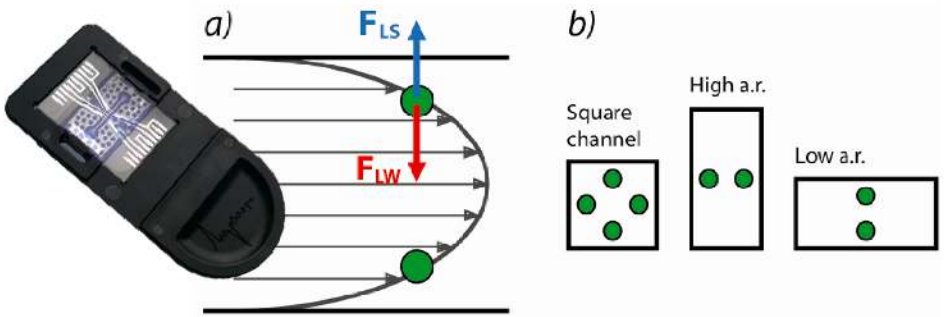
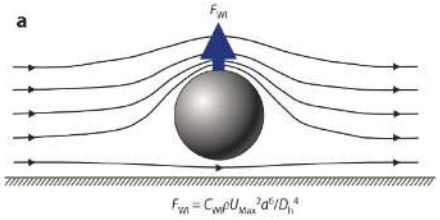
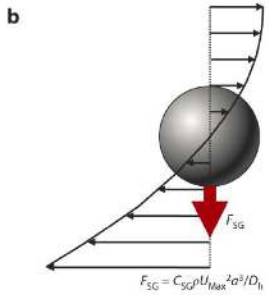


Figure 1: Particle inertial focusing in flow through straight channels. (a) The shear induced lift force (F_{LS}) and a wall induced lift force (F_{LW}) acting on a particle flowing in a microchannel. (b) Illustration of the cross-sectional equilibrium positions of particles flowing through different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

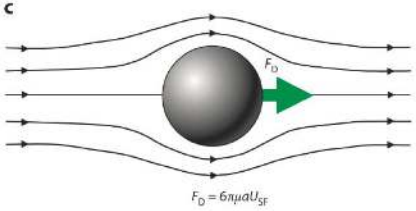
Wall-induced lift force



Shear-induced lift force



Viscous drag force

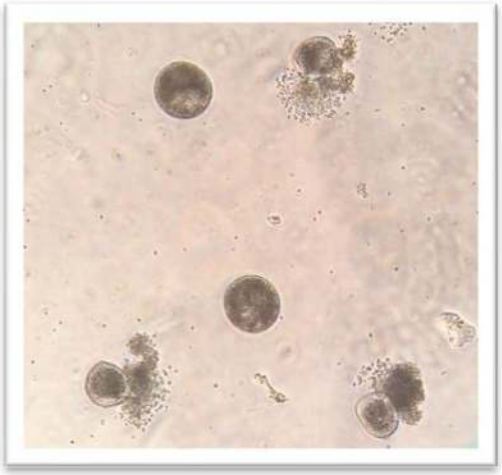


Adapted from Hansson et al. 2011

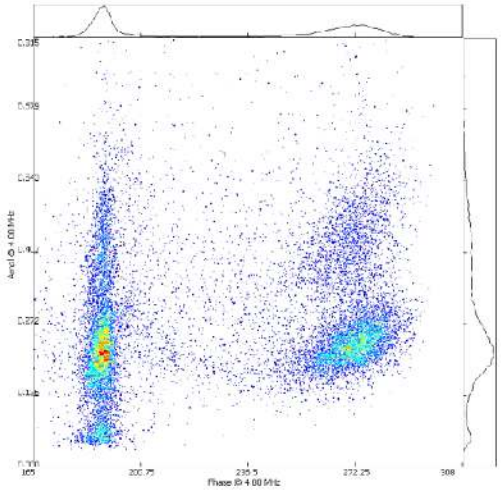
Bursting Cells

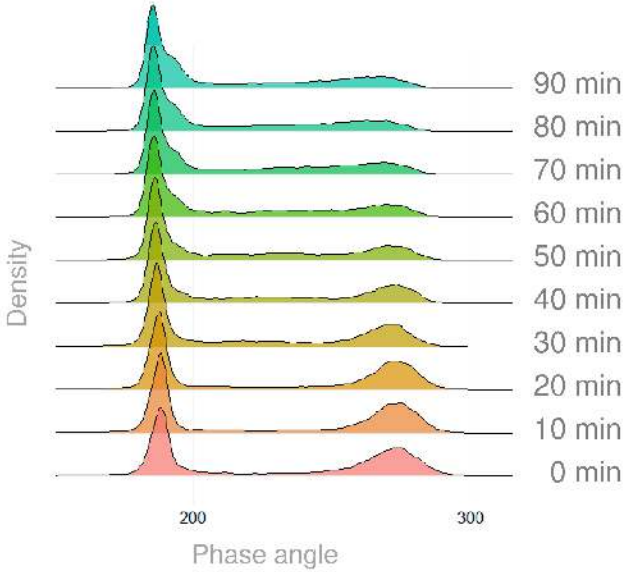
- Cell bursting after suspension in buffer, presumably due an osmotic effect
- In the plot, viable cells are moving towards the dead population. An intermediate cell population is visible.
- Prevention: Measuring right after sample preparation
- *Quick Guide*: Stability of pollen cells (website)

Bursting Pollen



90 min Time Lapse



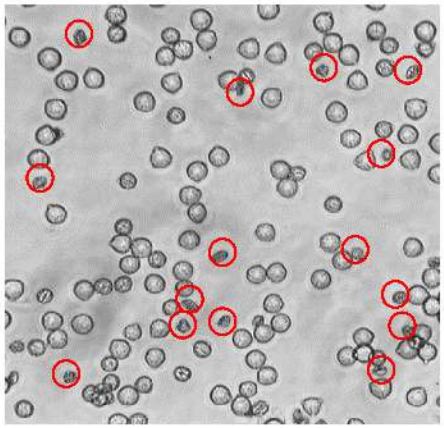


Going Beyond Viability

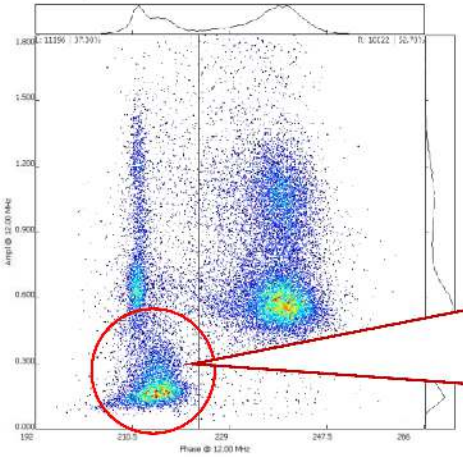
Small deformed cells

- For some species (e.g. tomato), a significant population of smaller deformed cells can be visible under the microscope and using the Ampha Z32
- Significance? Data Analysis?

Microscopy



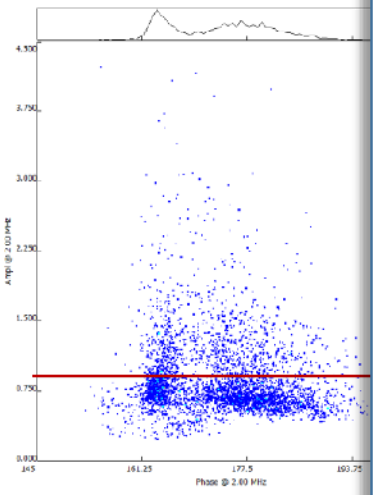
Ampha Z32

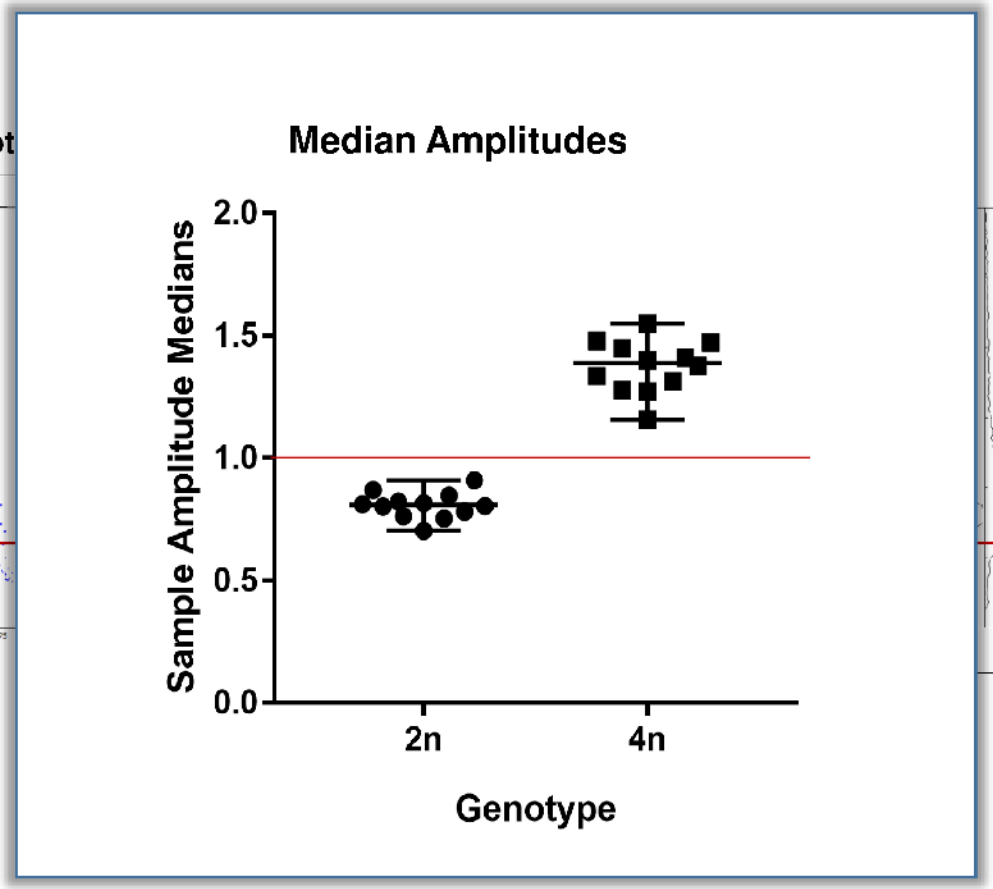


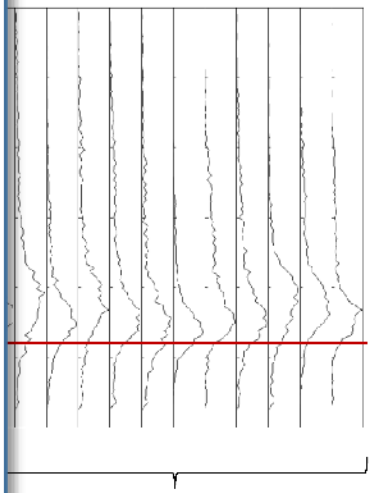
- How would you count them when doing microscopy?
- Would you classify these cells as dead, apply *Hide Cells,* or quantify them individually?

Ploidy using *Advanced Gate Statistics*

- Higher pollen ploidy is often associated with larger pollen grains
- Can we determine pollen ploidy by IFC?







Q&A

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