… Reinventing Single Cell Analysis

Pro Session

Amphacademy 2017

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Topics for today's pro session

- Sample preparation
- Data analysis
- Autosampler
- Tips and tricks for optimizing measurements
- Introduction of new features
- Support and maintenance
- Types of measurements
- Q&A

Impedance Flow Cytometry (IFC)

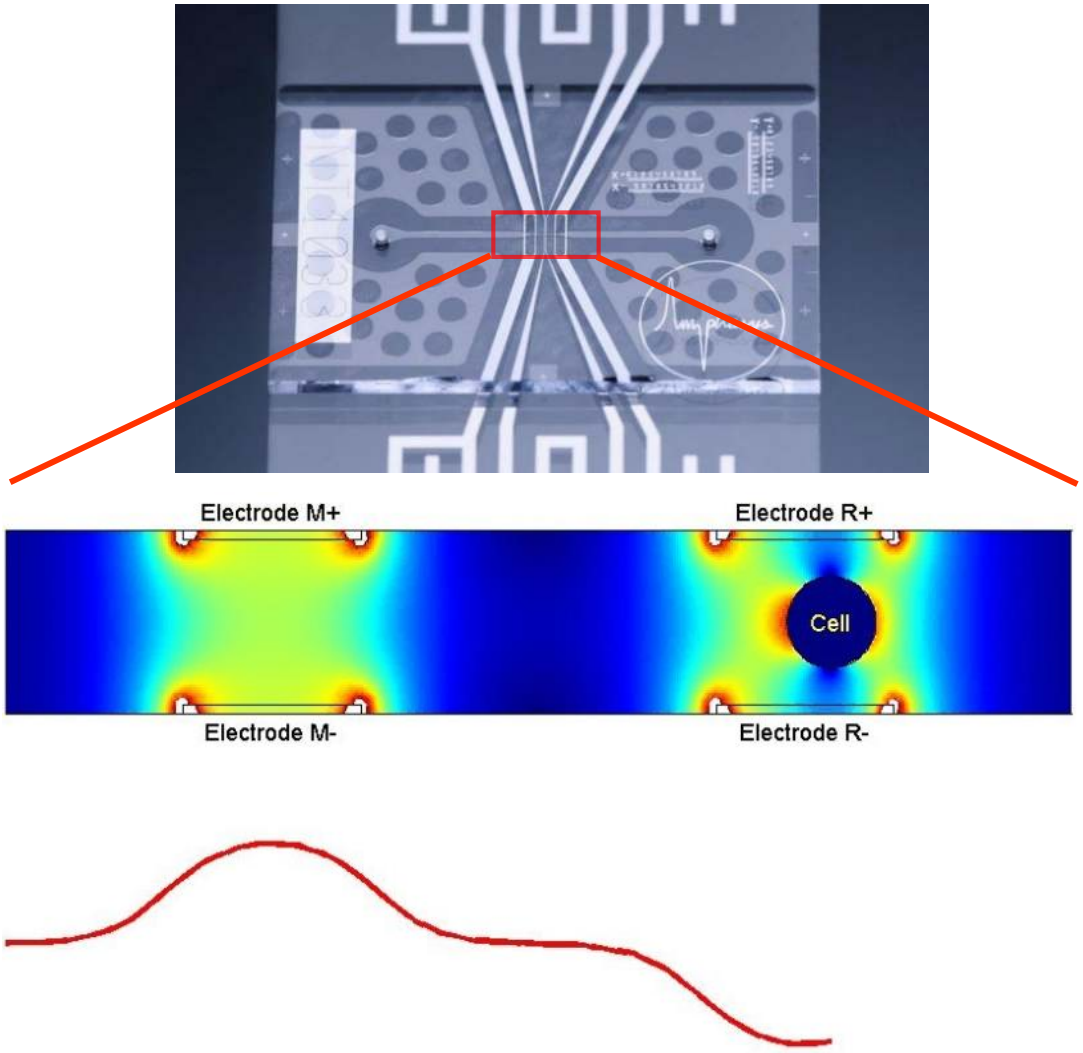
To replay the film please visit
www.amphasys.com/lab-on-chip_label-free

In this chip we measure changes of the electrical impedance of a fluidic medium when cells pass through the applied electric field.

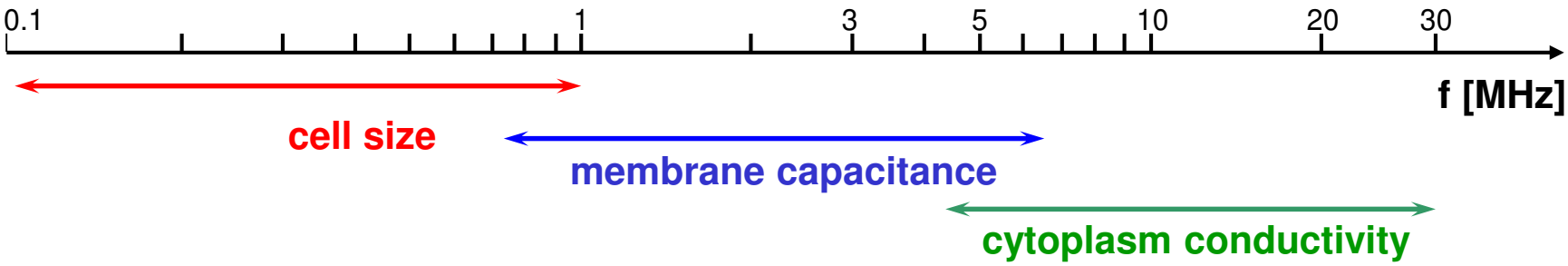
Impedance Measurement

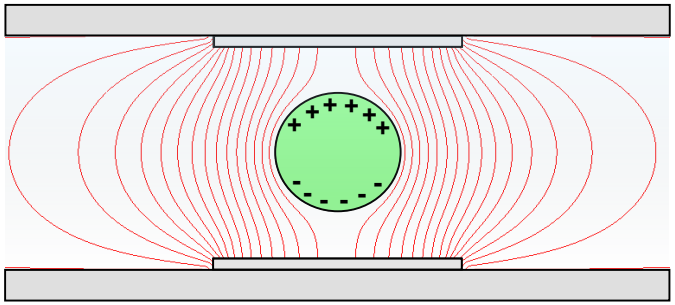
3 different pollen chips are available:

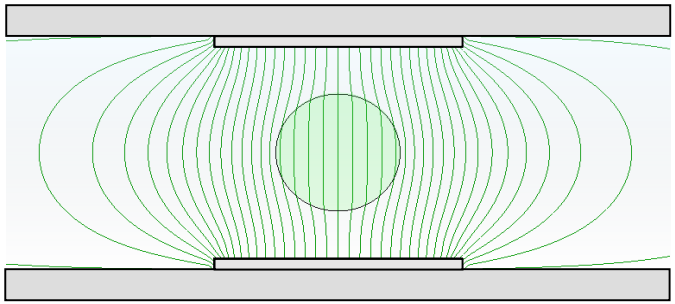
- F: 80µm channel
- D: 120µm channel
- E: 250µm channel

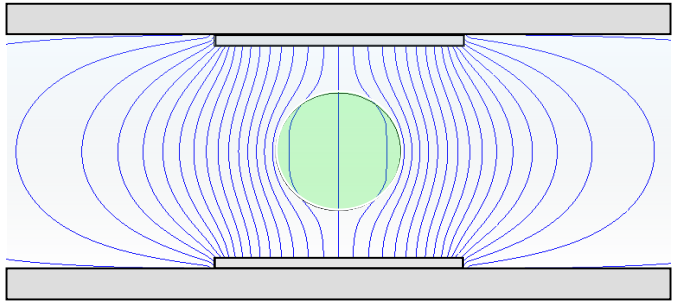


Technology – Multi-Frequency









1. Introduction – top secret

Disclamer:
this story around the presentation of the various aspects of the technology is made up ☺

A few years back, Marco and I started a tomato seed business...

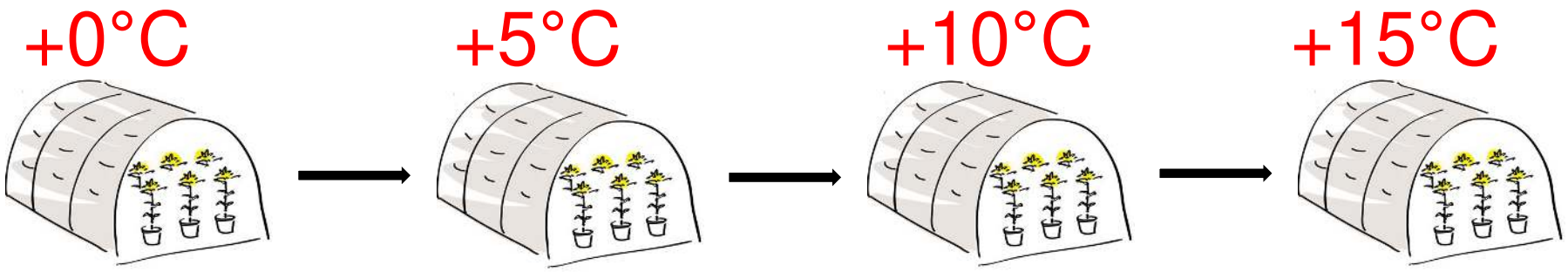
1. Introduction – top secret

Goal was to create a new tomato variety that could meet the challenges that global warming poses...

Overview of our master lines

ID	Code	Trait	Property	Origin
1	H0t	Heat resistant	Sometimes yields low producing tetraploids	Bred in-house
2	Gr8	Great taste	Only available as stored pollen	Acquired as pollen stock
3	Tuf	Tobacco Mosaic Virus resistance	Pollen tightly associated to anther	Acquired as plants
4	A-L0t	Great yield at ‘normal’ temperatures	Produces many pollen	Acquired as seed

Creation of the heat-resistant line



- The first selection steps were fully automated
  - Instrument capacity = 200 samples/day
  - We had 10 instruments with autosamplers = 2'000 samples/day
  - The initial screen took about 3 months

Autosampler

Pollen Analysis by Amphasys

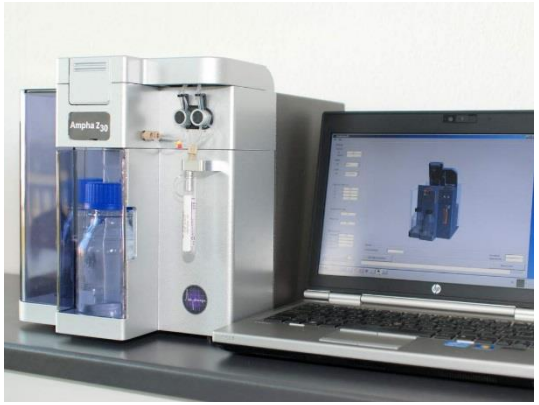
1. Pollen collection

- collect pollen

2. Sample preparation

- dispense pollen with buffer solution (on-site)
- filter to remove dirt

3. Measurement



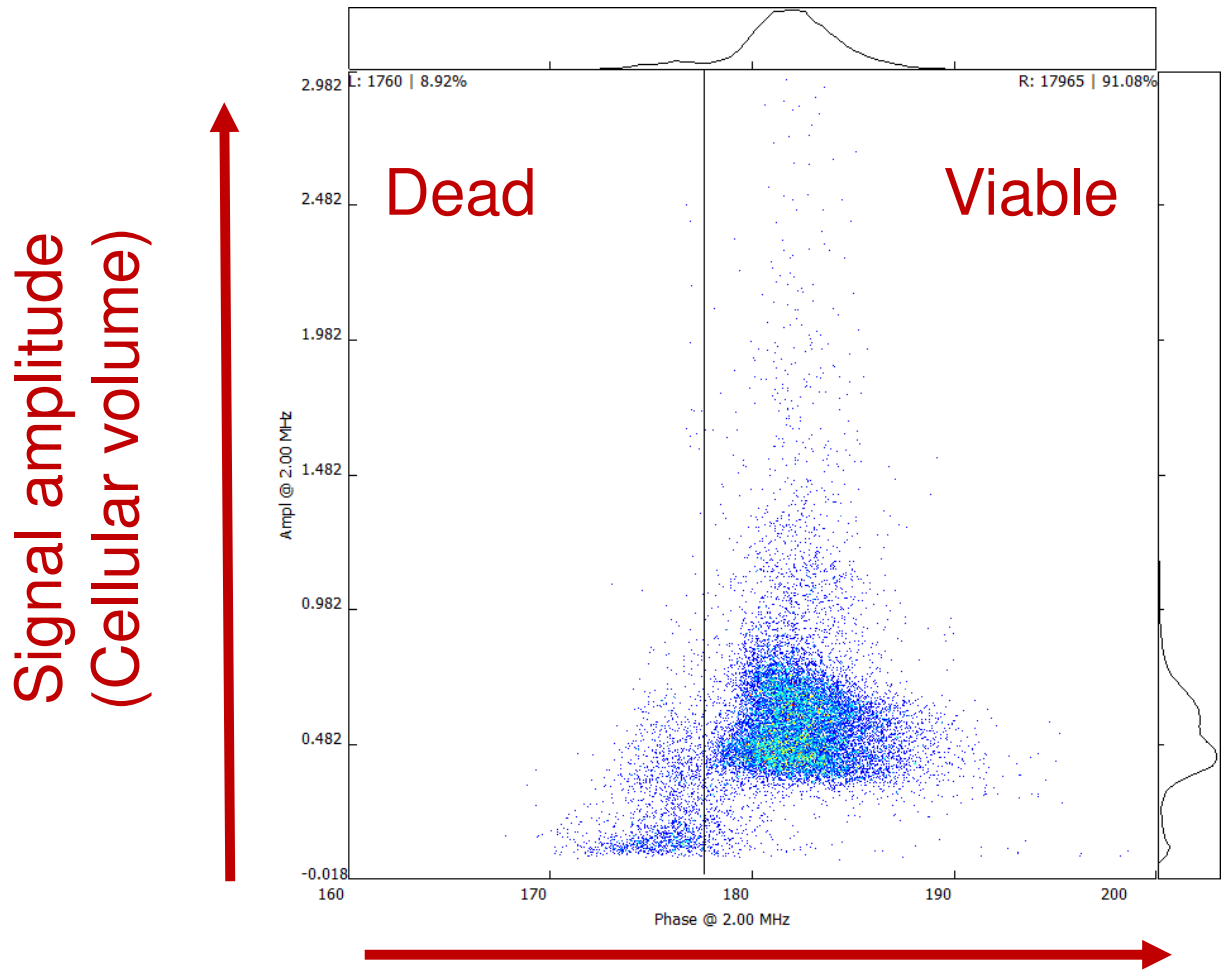
- measure (approx. 1 minute, on-site)
- analyze (software supported)

Heat inactivation (1)

Important points:

1. Heat inactivation can aid the correct placing of gates
2. Care should be taken not to change the concentration of the buffer
    1. Heat inactivate in a small drop of buffer
    2. Cool down the sample before opening the tube
    3. Dilute the sample after heat inactivation with additional buffer
3. Filter the sample to remove pollen aggregates

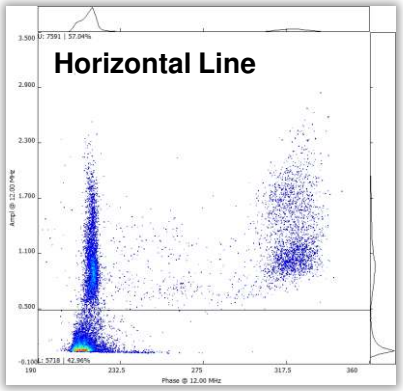
Basic plot properties

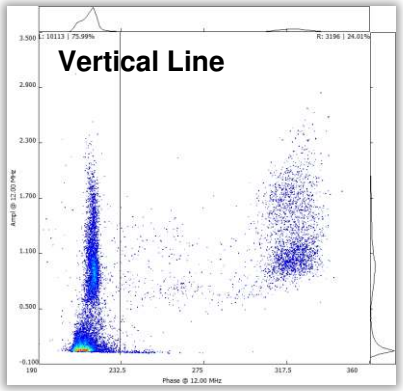


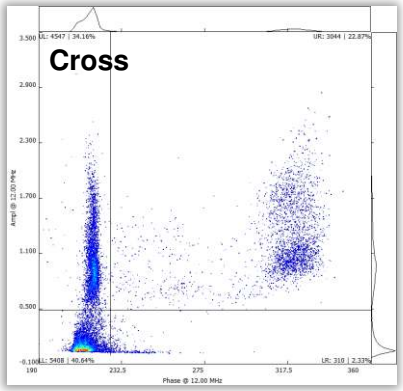
Data Analysis

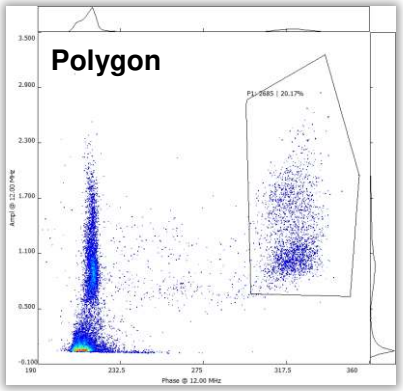
Important for proper data analysis:

1. Display the clouds such that the whole plotting area is used
2. Change the dot size for faint plots
3. Change the density plot coloring
4. Place a gate



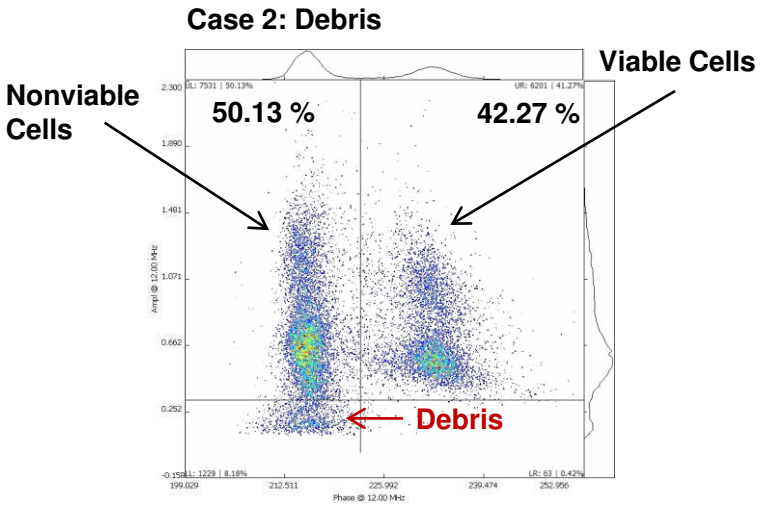


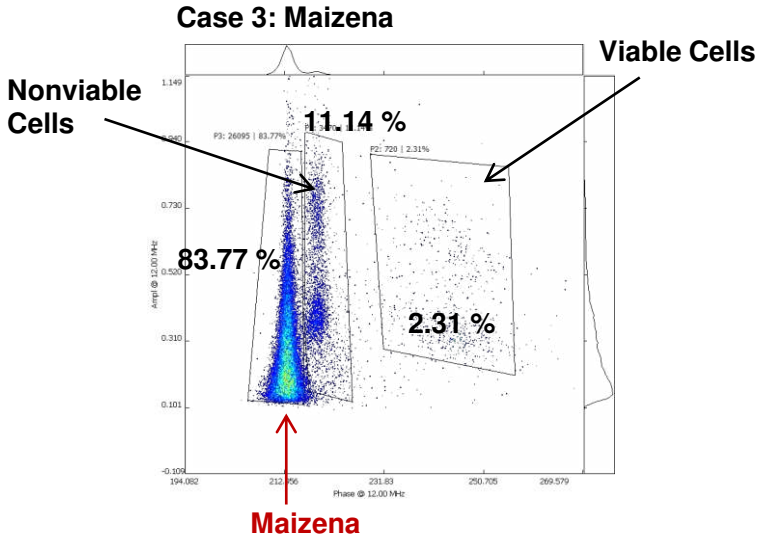




Data Analysis – Gating with other particles

- Depending on the type of sample, other types of particles than pollen cells can be present. In that case, polygon or cross gates are used to correctly quantify viable and nonviable cells.





$$V(\%) = \frac{41.27\ \%}{41.27\ \% + 50.13\%} \times 100\ \% = \mathbf{45.2}\ \%$$

$$V(\%) = \frac{2.31\ \%}{2.31\ \% + 11.14\ \%} \times 100\ \% = \mathbf{17.2}\ \%$$

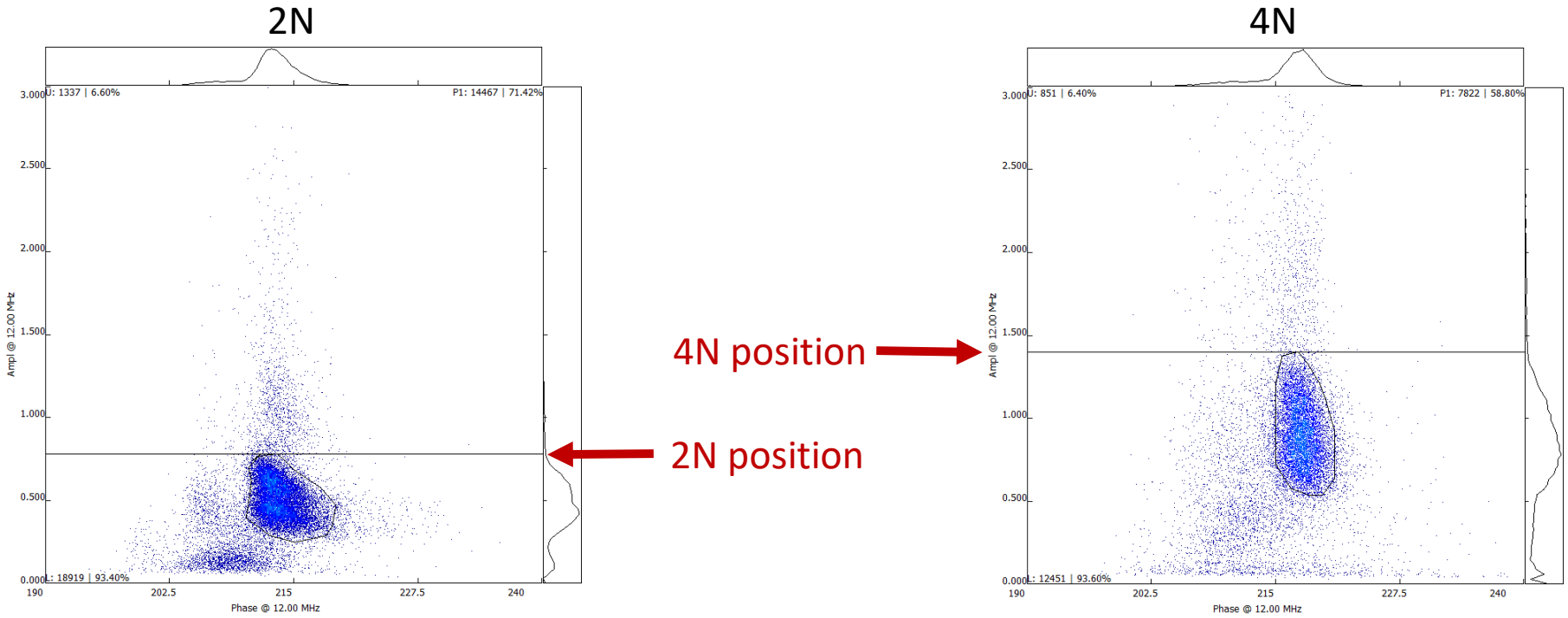
Measuring of the 4 master lines

ID	Code	Trait	Property	Origin	Pollen viability
1	H0t	Heat resistant	Sometimes yields low producing tetraploids	Bred in-house	

Besides viability, we are interested in the ploidy level of the progeny

Determining ploidy, in addition to viability

Ploidy is determined on the vertical axis, as it is a property of signal amplitude



Measuring of the 4 master lines

ID	Code	Trait	Property	Origin	Pollen viability
2	Gr8	Great taste	Only available as stored pollen	Acquired as pollen stock	

To measure stored pollen, we have to re-hydrate

Rehydration

Why is it important?

1. The technology works because living cells distribute their charge better than dead cells
2. This redistribution of charge happens in an aquatic environment

Measuring of the 4 master lines

ID	Code	Trait	Property	Origin	Pollen viability
3	Tuf	Tobacco Mosaic Virus resistance	Pollen tightly associated to anther	Acquired as plants	

To analyze contaminated pollen, we have to hide the debris

Measuring of the 4 master lines

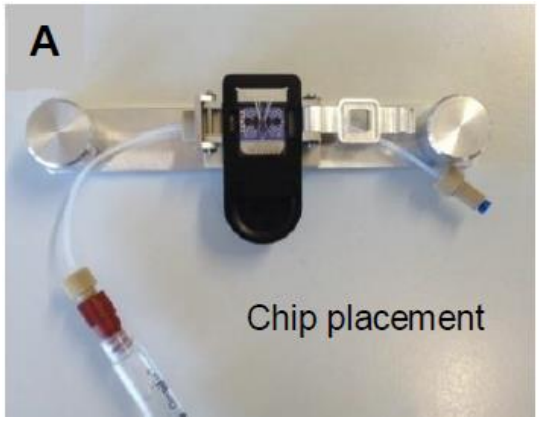
ID	Code	Trait	Property	Origin	Pollen viability
4	A-L0t	Great yield at ‘normal’ temperatures	Produces many pollen	Acquired as seed	

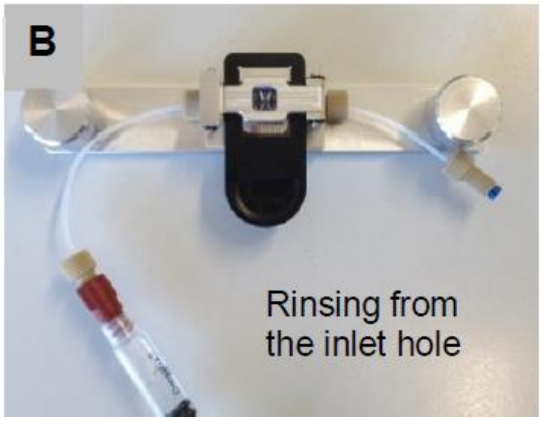
Do not overload your sample with pollen, a chip blockage may occur

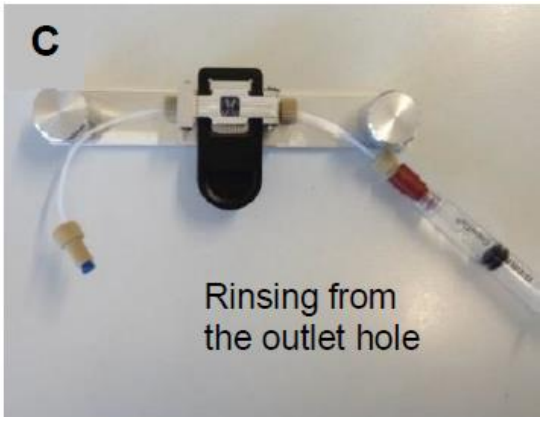
Chip unblocking tips (1)

When a blockage occurs:

1. Flow speed goes down or tubing pops off
2. Stop the measurement before opening the lid
3. Open lid and determine position of blockage (usually after the inlet port)
4. Set-up the chip washing station accordingly



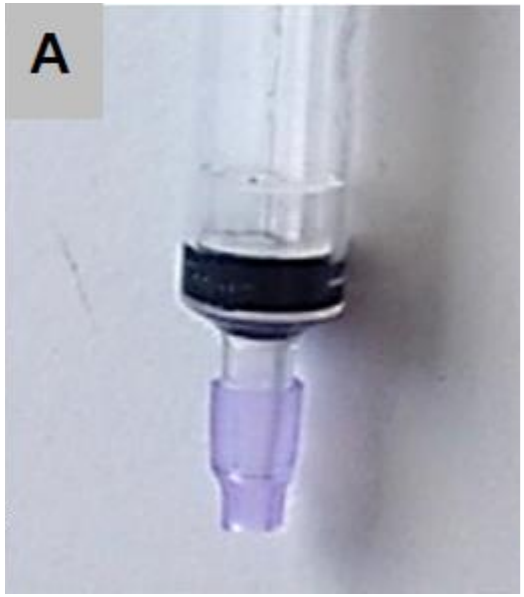


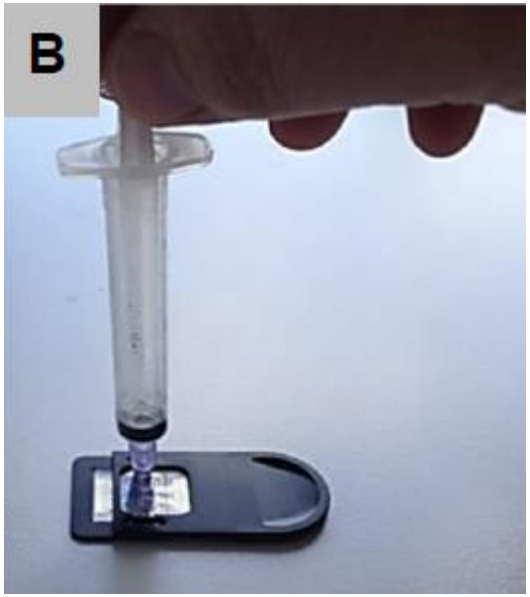


Chip unblocking tips (2)

If the blockage does not get resolved with the chipwash station, then:

1. Apply syringe with silicone tip directly to the chip
2. Push water or air from the outlet port
3. Pull water or air from the inlet port





Chip unblocking tips (3)

If the blockage does not get resolved with either method, then:

1. Dry the chip overnight and repeat with wash station
2. Place the chip in an ultrasonic bath with clean water for one minute
3. Call support ☺

Take-home message:

keep calm and be gentle with the chip.

Our support and maintenance plan

1. Free remote technical support
2. One on-site visit per year
   - Maintenance, including service materials
   - Expert training
3. Materials for quarterly maintenance
4. Q&A workshop
5. Unlimited consumables
   - Buffers
   - Chip replacements
6. Replacement instrument in case of breakdown
7. Extended warranty during the agreement
8. Unlimited Amphacademy participations!! ☺

Overview of data generated with the Z32

Pollen viability

Microspore maturation

Pollen germination

Pollen counting

Pollen concentration

Pollen ploidy

Interesting links

- Species list (always expanding): http://www.amphasys.com/sites/default/files/170123_AmphaFluid_Buffer_Recommendations.pdf
- Pollen paper: http://www.amphasys.com/sites/default/files/Heidmann%20et%20al%202016.pdf
- General references to impedance flow cytometry: http://www.amphasys.com/cell-analysis-pollen-viability#Analysis of other single cells
- In-field use: http://www.amphasys.com/sites/default/files/Amphasys_in-field_Wheat_Pollen_Analysis_by_Bayer_CropScience.pdf
- Our youtube channel (from sample prep to automated analysis): https://www.youtube.com/channel/UCRnK6JcIZdMmfX7z8lTI2Iw

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