How to collect and prepare the perfect sample for your application

Amphacademy
September 14th 2018

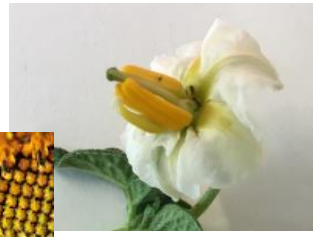
Content

Have you ever wondered at what time point to collect your samples, how many and where?

In this session we talk about different sources of variation and how to sample correctly in order to have a representative result.

On top of that, we'll show you how to optimize sample preparation to reduce debris, chip cloggings and measurement bias.

Optimizing Sampling


- Do you know your plant ?
  - Flower structure
  - Flowering time point
  - Amount of pollen per anther or flower
- A successful measurement starts with successful sampling. *Garbage In - Garbage Out.*
- Do not compare apples with oranges. Do not draw conclusions from a single sample taken from a highly variable source...
  - Variability within a plant, flower, tassel...
  - Variability during the day
  - Different developmental stages
  - Environmental factors
- Properly rehydrate / equilibrate after freeze-storage
  - Frozen cells can show a similar behavior as dead cells
- Work with standardized protocols
  - Pollen source and collection method

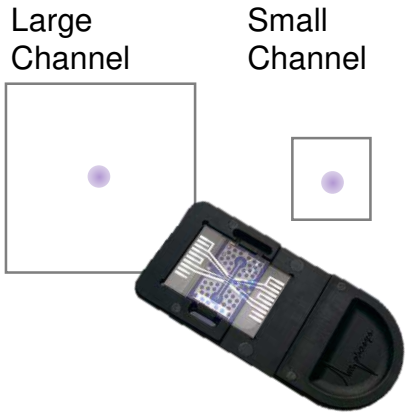
Optimizing Sample Preparation I

- Work with standardized protocols if you want to compare results
  - Buffer, Chip Type, Settings, Filter Size, Sample Preparation Methodology
- Use recommended AmphaFluids for your pollen or perform test protocols to find the optimal buffer
- Know the stability of the cells in this buffer – Test it !
- Add 0.05 % Tween 20/80 to the buffer aliquot for hydrophobic or sticky cells
- Check the buffer quality
  - Clear solution, no microbial growth
  - Buffer equilibrated to room temperature
- Rehydrate samples if they were dehydrated, check optimal time and condition (temperature x humidity)
- Use natural dead controls if possible (old flowers or store pollen at room temperature or any other incubator)

The most important factors affecting the resolution between cell subpopulations

Chip

Cell-to-channel size ratio



- 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)

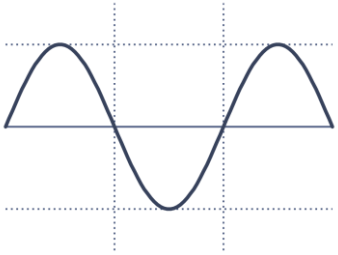
Buffer

Composition

- 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

Electric Field

Frequency

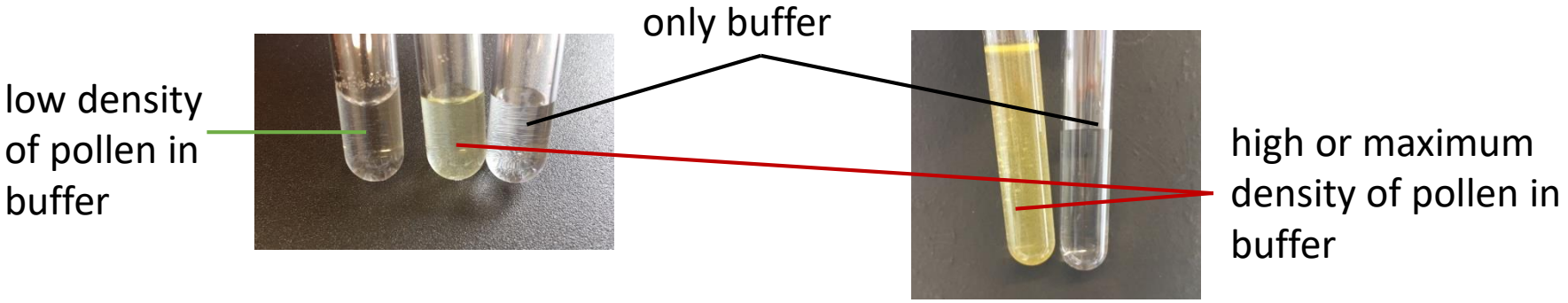


- The signal-to-noise ratio decreases for increasing numbers of frequencies simultaneously applied
- 2 frequencies are recommended for most applications
- Changing the triggering frequency (by default the first frequency) typically requires adaptation of other parameters as well (Triggering source, Triggering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

Optimizing Sample Preparation II

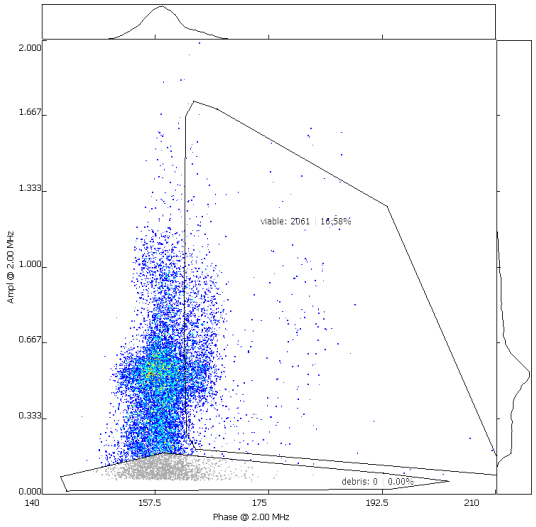
- Work best with collected pure pollen > isolated anthers > whole flowers/buds
- Treat your cells gently
  - Vortex anthers in buffer to release mature pollen
  - Do not forcefully squeeze anthers with a pistil in order to release pollen
  - option: cut the anthers with a scalpel and vortex in buffer

- 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...
  - From a open field you cannot avoid all debris – correct with software
- Use appropriate cell concentrations to minimize the risk of chip clogging while still being able to acquire high cell numbers in a short time

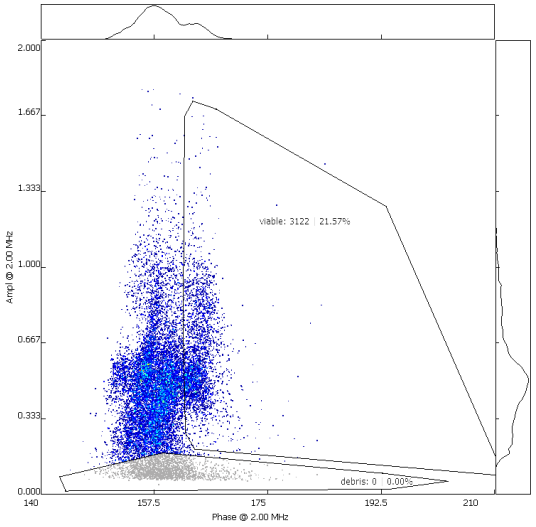


Brassica napus – single flowers of different stage in AF7

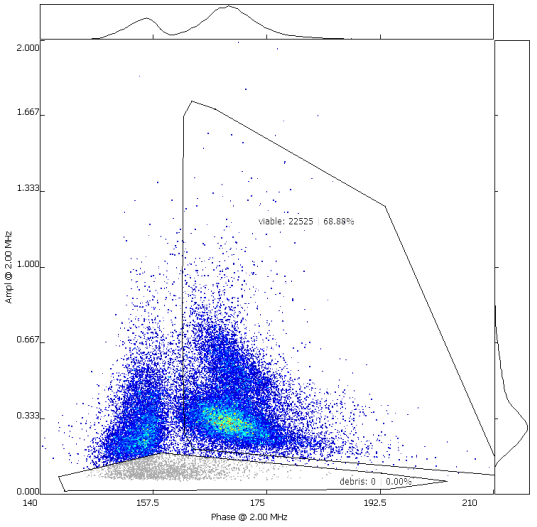
sample 1



old open flower

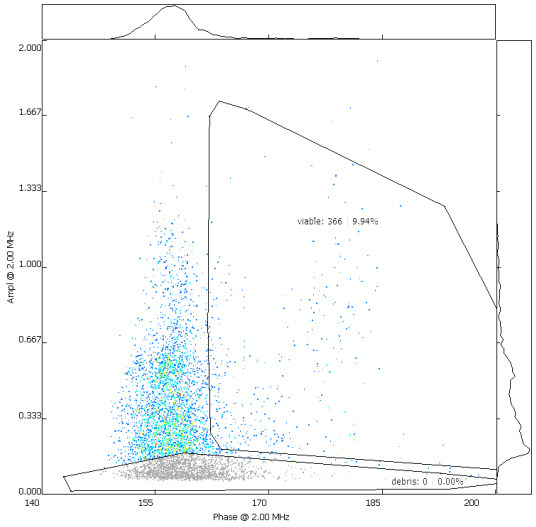


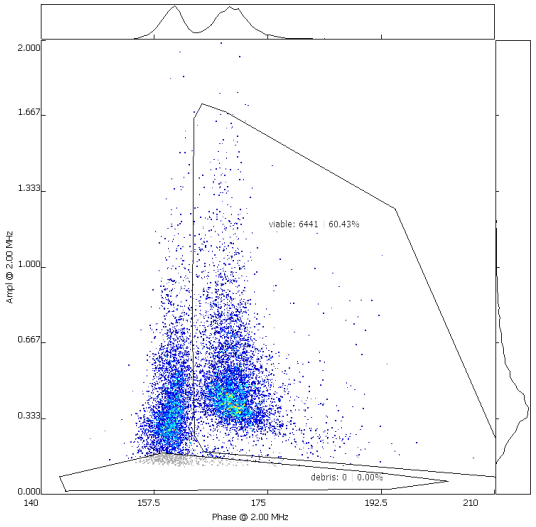
open flower

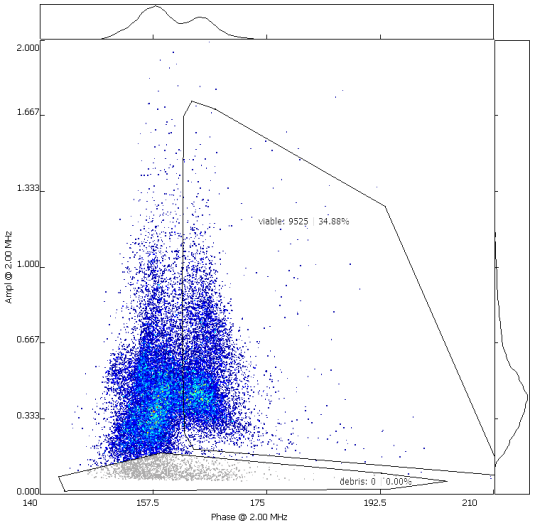


Opening flower

sample 2





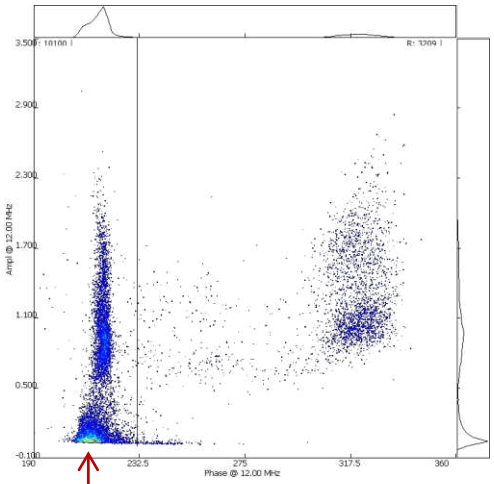


Hide Cells to Correct for Debris

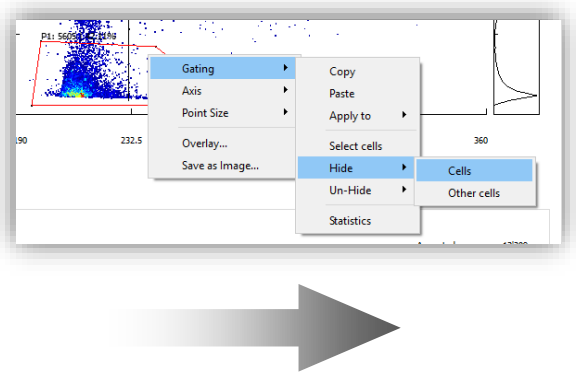
- *Hide Cells* to exclude debris from the analysis
- Hide *polygon gate* content or everything around the polygon
- Apply *Hide Cells* gate to other measurements as usual

Viability before correction

24.1 %

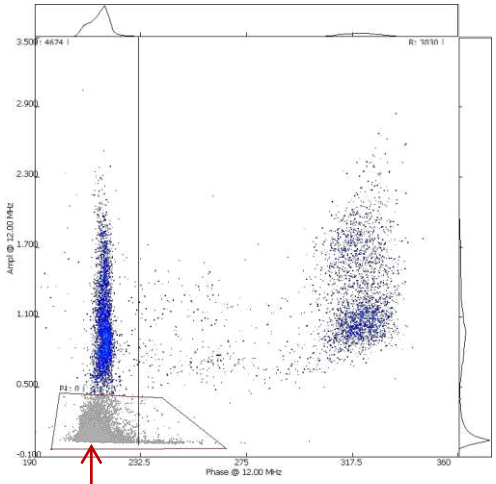


Debris



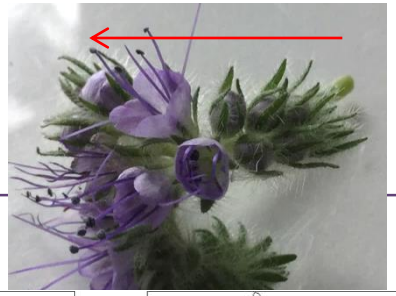
Viability after correction

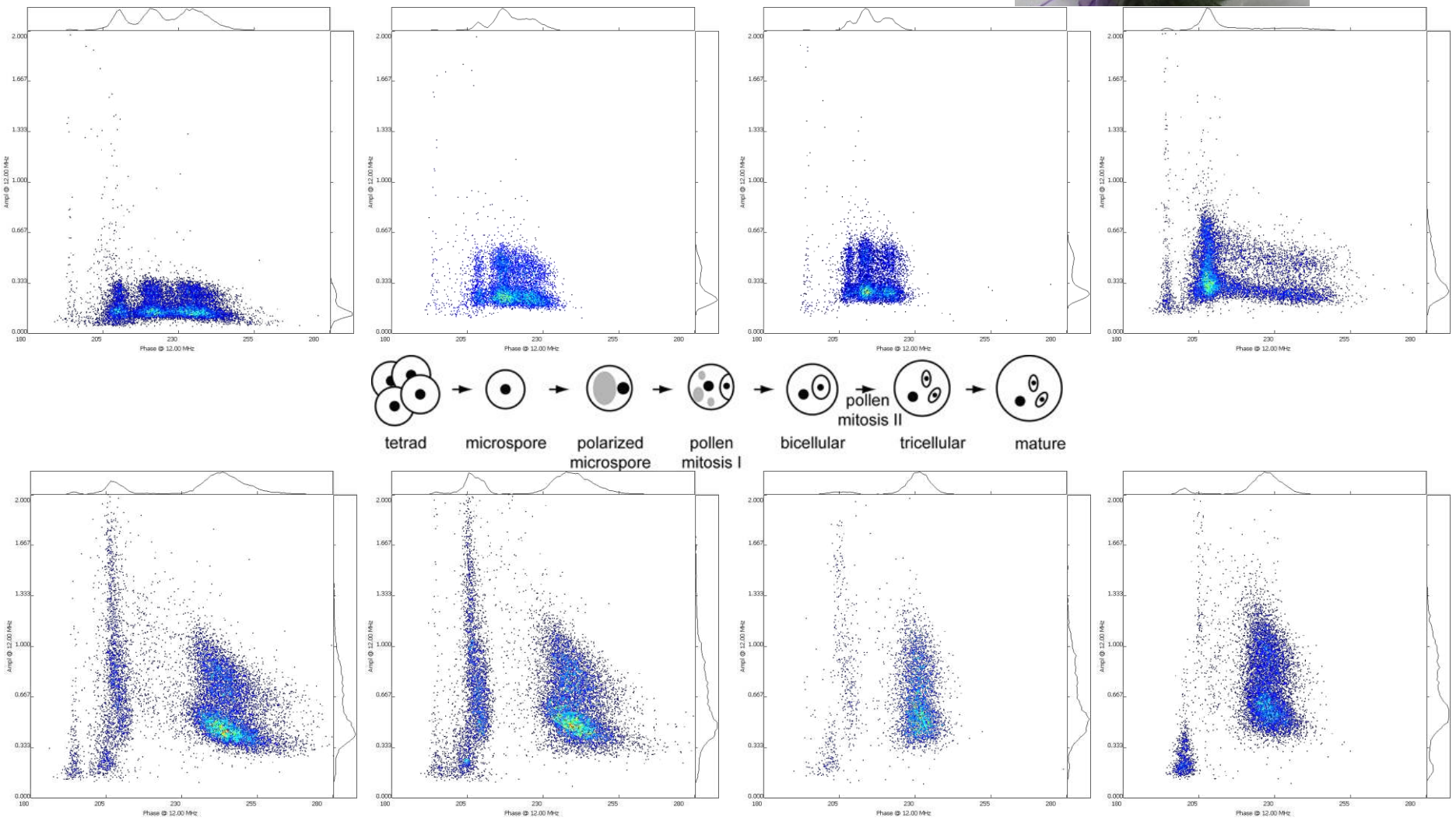
39.4 %



Hidden Debris

Pollen development – *Phacelia*





Your Contacts

Grit Schade, PhD
Senior Application Manager
Tel: +41 41 541 91 22
grit.schade@amphasys.com

Silvan Kaufmann
Application Scientist, MSc Biomedical Engineering ETH
Tel: +41 41 541 91 22
silvan.kaufmann@amphasys.com

Support
support@amphasys.com

Amphasys AG
Technopark Lucerne
Platz 4
CH-6039 Root D4
Tel: +41 41 541 91 20
www.amphasys.com