QUICK GUIDE: STABILITY OF POLLEN CELLS

Introduction

In Impedance Flow Cytometry (IFC), pollen grains are suspended in a measurement buffer during the measurement. The AmphaFluid measurement buffers were carefully developed in order to keep the cells stable until they are measured. For certain applications (e.g. using the autosampler), the cells must be kept stable for longer periods of time. For certain species and developmental stages, it has been observed that pollen grains lose their viability or burst after a while when suspended in buffers. Unless the samples are measured immediately after preparation, the stability of the cells should be tested to prevent biased experiments.
The following protocols can be used to determine the maximum suspension time of pollen grains using IFC and microscopy. It is recommended to follow both procedures (A and B).

Materials

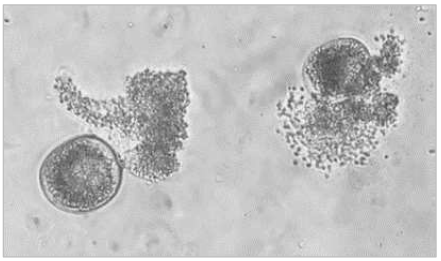
- Pollen sample
- Measurement buffer, chips and filters
- Sample preparation materials
- Measurement template
- 50 ml Falcon tube
- Microscope and a microscopy slide or 12 well plate

Protocol for the determination of the stability of cells in the buffer

A) Viability measured by IFC

1	Prepare Instrument	• Prepare your Ampha Z32 instrumentation according to the instructions described in the ***Startup Procedure Quick Guide***.
2	Prepare Samples	• Collect a pollen sample with enough material for about 10 measurements in a 1.5 ml Eppendorf tube. • Add 1 ml measurement buffer to the tube, disperse the cells and filter the sample into a 50 ml Falcon tube. ***TIP:*** *Recommended pollen quantities and sample preparation methodology can be found in the* ***Sample Preparation Quick Guide.*** • Dilute the sample using 20 ml measurement buffer. • Note the time, e.g. 3.00 pm.
3	Measure First Sample	• Immediately take a subsample and measure it.
4	Measure Other Samples	• Take other subsamples after 5, 10, 15, 20, 30, 45 and 60 minutes (or your own relevant time periods) and measure them. ***TIP:*** *Make sure you homogenize the original sample well before removing subsamples.*
5	Repetition	• Repeat the procedure for other samples if needed.
6	Data Analysis	• Determine the cell viability by gating. • Create a .csv report. ***TIP****: Tips and tricks for data analysis are shown in the* ***Data Analysis Quick Guide****.* • Plot the measured cell viability vs. suspension time, e.g. using Microsoft Excel or R. • The time for which the maximum measured viability is maintained is the maximum recommended suspension time. ***TIP****: If the cells are not sufficiently stable for your application, don't hesitate to contact Amphasys Support.*

B) Bursting observed by microscopy

1	Prepare Sample	• Prepare a 1 ml pollen sample according to your sample preparation methodology. • Note the time, e.g. 3.00 pm. ***TIP:*** *Recommended sample preparation methodologies can be found in the* ***Sample Preparation Quick Guide****.* ***TIP:*** *The cell concentration can be higher than in regular IFC measurements.*
2	Microscopy	• Shake the sample well and add 100 µl on a microscopy slide or add the whole sample into a well of a 12-well plate. • Let the cells sediment and check whether you can observe cell bursting during the relevant time period. • Note the time after which a considerable portion of the cells has burst. Burst Wheat Pollen 
3	Repetition	• Repeat the procedure for other samples if needed. ***TIP:*** *Cell bursting depends on the species and developmental stage of the pollen among other factors.*
4	Conclusion	• Pollen should only be suspended for as long as no substantial cell busting can be observed.