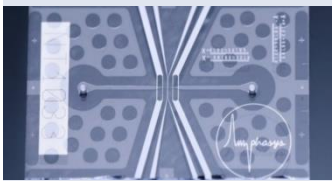
APPLICATION NOTE

SOLANUM TUBEROSUM
POLLEN VIABILITY AND DEVELOPMENT

Pollen viability and development plays a crucial role in breeding and seed production processes, for the development of new lines and quality improvement. With the Amphasys Impedance Flow Cytometer pollen stages and many other cells can be individually analyzed in the field or laboratory. Amphasys protocols enable quick and accurate pollen quality control and comparisons between samples in order to identify the best material for breding development, pollination and to optimize storage procedures.

AmphaZ32 Impedance Flow Cytometer

- Rapid
- Accurate
- Reproducible
- Label-free
- Portable for on-site analysis



STRAIGHTFORWARD 3-STEP WORKFLOW

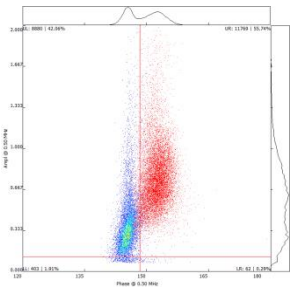
1 Pollen Resuspension → 2 Filtration and Dilution → 3 Analysis

POTATO POLLEN VIABILITY

- Measurement of the pollen of one anther at 2 MHz and 12 MHz frequency
- Use of an old flower or heat-inactivated sample to gate non-viable population and debris

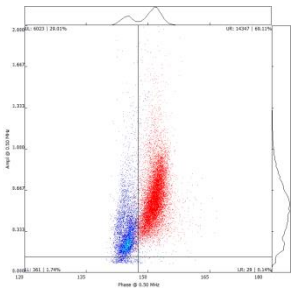
Line 1: flower open

55.7 % Viability



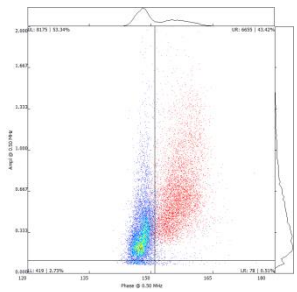
Line 1: flower closed

69.1 % Viability



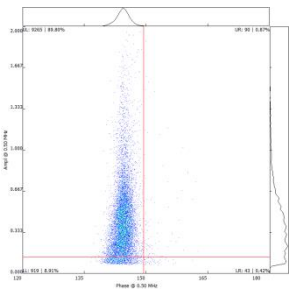
Line 2: flower open

43.4 % Viability



Line 2: old flower

0.9 % Viability



Takeaways

- Control and selection of right pollen developmental stages for breeding
- Very precise quantification of pollen viability and identification of high quality pollen samples, control of CMS lines
- Optimized timing of pollen harvest
- Very high repeatability

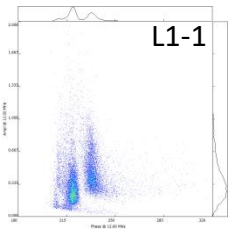
Sample Preparation

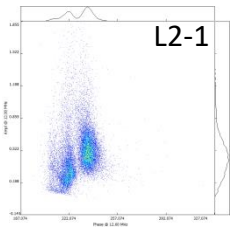
- Dissolve freshly collected pollen anthers or crush a flower bud in AF6 buffer
- Filtration using a 50 µm filter into a FACS tube
- Dilution with AF6 buffer, if necessary
- Stored and frozen pollen should be rehydrated before measurement

POTATO POLLEN DEVELOPMENTAL STAGES

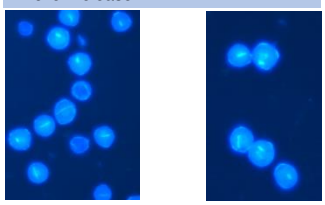
Impedance Flow Cytometry
Measurements at 12 MHz from different plant lines shown

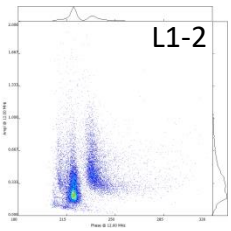
Microscopy
EtOH + acetic acid fixation
+ DAPI staining

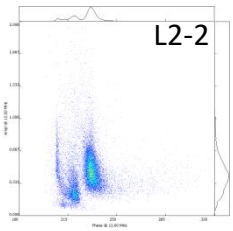




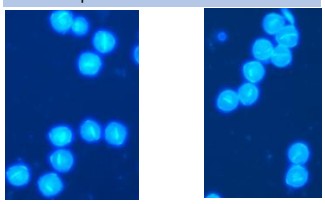
Pollen release

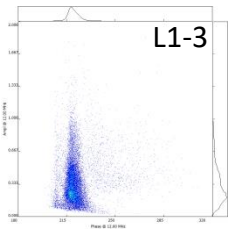


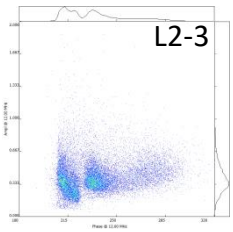




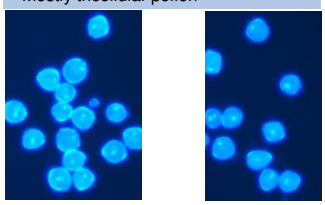
Mature pollen

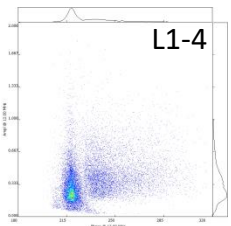


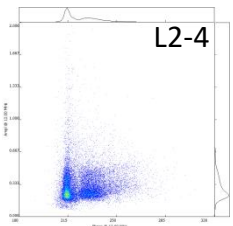




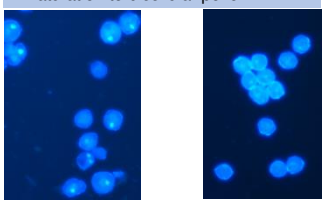
Mostly tricellular pollen

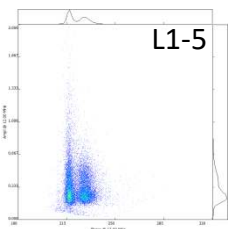


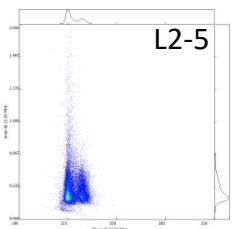




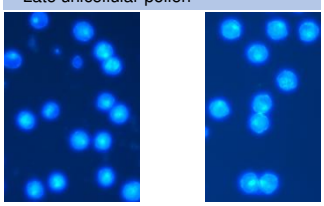
Maturation to bicellular pollen

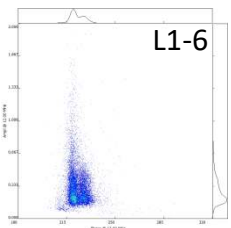


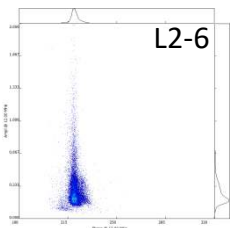




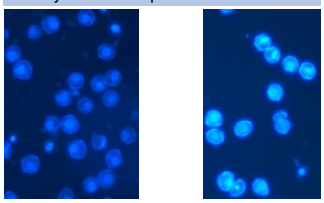
Late unicellular pollen

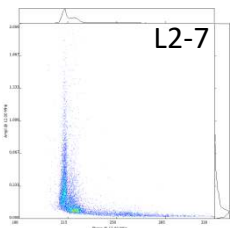




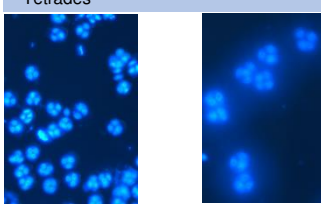


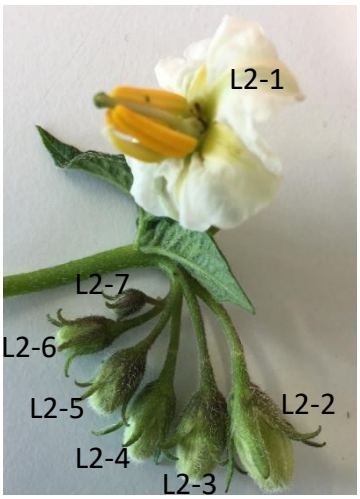
Early unicellular pollen





Tetrades





Analysis

- Impedance at 2 MHz is suitable for viability and 12 MHz for developmental stage analysis
- Identification of non-viable population and debris
- Gating and reporting

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