APPLICATION NOTE

PALM POLLEN VIABILITY

Palm pollen viability is crucial for successful pollination. Using Impedance Flow Cytometry, palm pollen viability can be measured very rapidly in the field. In addition to fresh samples, the quality of shipped or stored pollen can be quantified in order to prevent the use of lowquality pollen. For all that, Amphasys provides a simple workflow from sample preparation to data analysis. Use your measurement results to improve your processes!

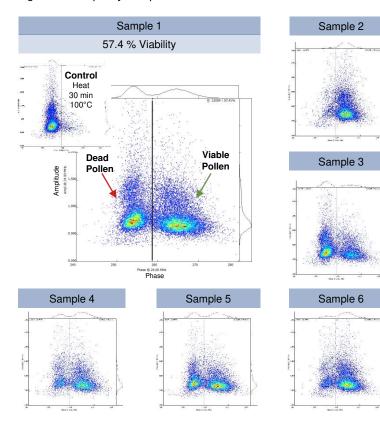


STRAIGHTFORWARD 3-STEP WORKFLOW



POLLEN VIABILITY TESTING

Oil palm pollen samples from different plants were stored at -20°C and underwent various numbers of freeze-thaw cycles. After thawing at room temperature, the samples were rehydrated in a humid environment for 1 h. After rehydration, minute amounts of pollen were suspended in measurement buffer, filtered and measured. As a negative viability control, one pollen sample was heat-treated for 30 min at 100 °C. This sample was used to determine a gate to identify the dead pollen. This protocol allowed a rapid discrimination between high and low quality samples.



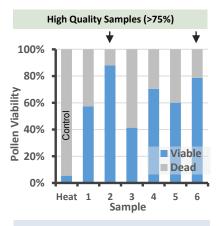
Ampha Z32 Impedance Flow Cytometer

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



IDENTIFICATION OF HIGH QUALITY POLLEN

Comparison of pollen sample viabilities right after collection, after storage or shipping to select top quality samples for pollination with a superior viability.

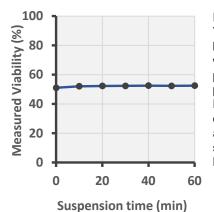


Takeaways

- Precise and rapid quantification of pollen viability and identification of high quality pollen samples
- High measurement repeatability
- Monitoring of pollen quality after storage or shipping
- Optimization of storage and rehydration steps
- Mixing of pollen samples to achieve target viability

POLLEN STABILITY IN MEASUREMENT BUFFER

Direct suspension of palm pollen in aqueous solutions is difficult, owing to the hydrophobic nature of the particles. Oil palm pollen tends to aggregate and adhere to tubing material. The use of detergents leads to uniform distribution of pollen particles in solution and therefore prevents sample loss by filtration and improves concentration accuracy.



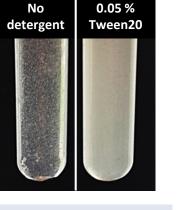
Ensuring pollen stability

The stability of oil palm pollen in AF6 buffer supplied with 0.05 % Tween20 was assessed by preparing a palm pollen suspension and measuring pollen viability over the course of 1 h. No decrease in viability could be observed. Therefore, it can be assumed that oil palm pollen suspensions remain stable for at least 1 h.



Improving pollen suspension

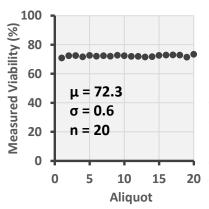
The effect of detergents on palm pollen suspension is shown in the figure below. Equal amounts of pollen were added to two 4 ml round bottom tubes and suspended with either AF6 measurement buffer or AF6 measurement buffer containing 0.05 % Tween20. The use of detergents prevents the formation of pollen aggregates, adhesion to the surface tube and quick accumulation of large particles at the bottom. Well dispersed pollen suspension can be obtained.



Sample Preparation

- Suspension of stored or freshly collected pollen in AmphaFluid AF6 buffer with 0.05 % Tween20
- Filtration using a 100 µm filter

HIGH REPEATABILITY



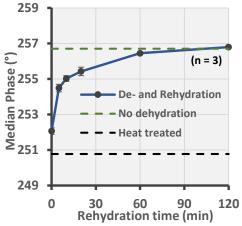
Repeatability

Pollen measurements using the Ampha Z32 Impedance Flow Cytometer show an extraordinarily high repeatability that can be attributed to the accurate electric measurement principle.

The measurement repeatability was quantified with measurements of 20 aliquots of one oil palm pollen sample under identical conditions. A mean viability of 72.3 with a standard deviation of only 0.6 % was obtained.

POLLEN QUALITY CONTROL

Pollen producers often need to store and transport pollen. Insufficient quality control can lead to low pollination success and thus commercial loss. The Ampha Z32 Flow Cytometer is the basis for optimal process monitoring and quality insurance.



Pollen Rehydration

After drying pollen samples at 10 % humidity for 1 h, the samples were rehydrated for various times in a humid environment. The median impedance phase was used as a measure of the rehydration status. As a control, median phases of a heat treated sample and a sample without dehydration were determined.

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