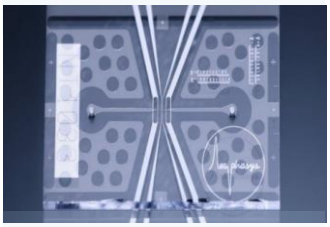
APPLICATION NOTE

BRASSICACEAE POLLEN VIABILITY AND POLLEN DEVELOPMENT

Pollen viability and development play a crucial role in breeding and seed production processes, for the development of new lines and quality improvement in Brassicaceaes. With the Amphasys Impedance Flow Cytometer (IFC) pollen stages and many other cells can be individually analyzed in the field, greenhouse or laboratory. Amphasys protocols enable quick and accurate pollen quality control and comparisons between samples in order to identify the best material for breeding development, DH-protocols, pollination and to optimize storage procedures. Use your measurement results to improve your processes!

AmphaZ32
Impedance Flow Cytometer

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

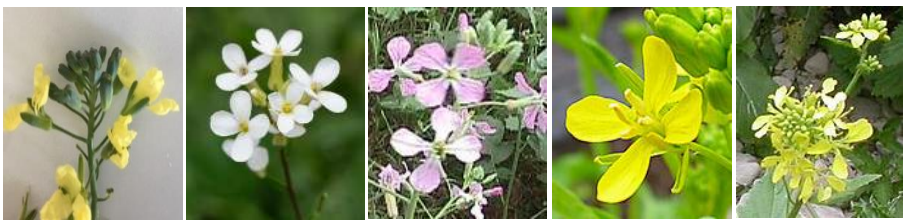


Takeaways

- Control and selection of right pollen developmental stages for breeding, DH production
- Very precise quantification of pollen viability and identifi-cation of high quality pollen samples,
- Optimized timing of pollen harvest
- Very high repeatability

Straightforward 3-Step Workflow

1 Pollen Resuspension → 2 Filtration and Dilution → 3 Analysis

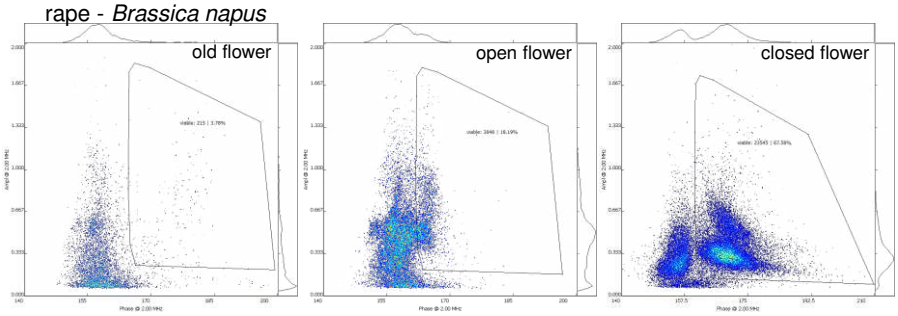


BRASSICACEAE - POLLEN VIABILITY

Pollen viability is a key factor for successful pollination and influenced by many factors, such as temperature, humidity and use of pesticides. Pollen can be mechanically extracted from anthers by squashing, or shed pollen can be suspended directly in measurement buffer.

- Measurement of the pollen of one flower at 1 or 2 MHz and optionally at 12 MHz frequency.
- Use of an old flower to gate non-viable population.
- *Brassicaceae* flowers include anthers of various pollen development stages.
- Crush a flower bud, anthers or freshly collected pollen sample in AF7 buffer for mature pollen or AF3 for microspores
- Filtration using a 50 µm filter into a FACS tube
- Dilution with AF7 resp. AF3

rape - *Brassica napus*

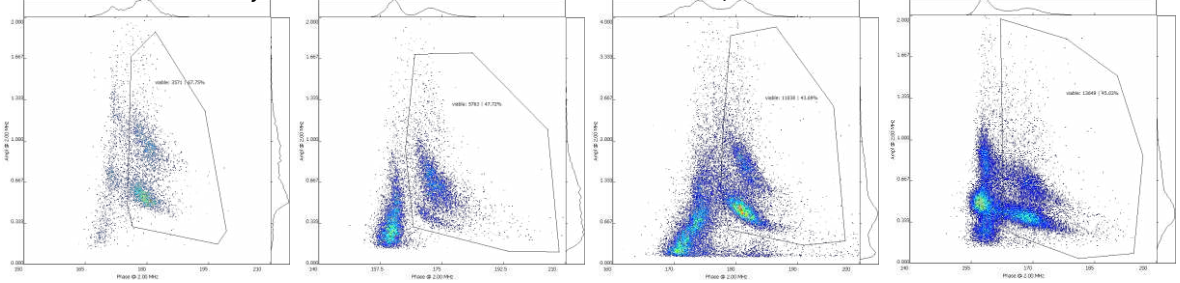


cauliflower – *Brassica oleracea botrytis*

broccoli – *Brassica oleracea italica*

radish- *Raphanus sativus*

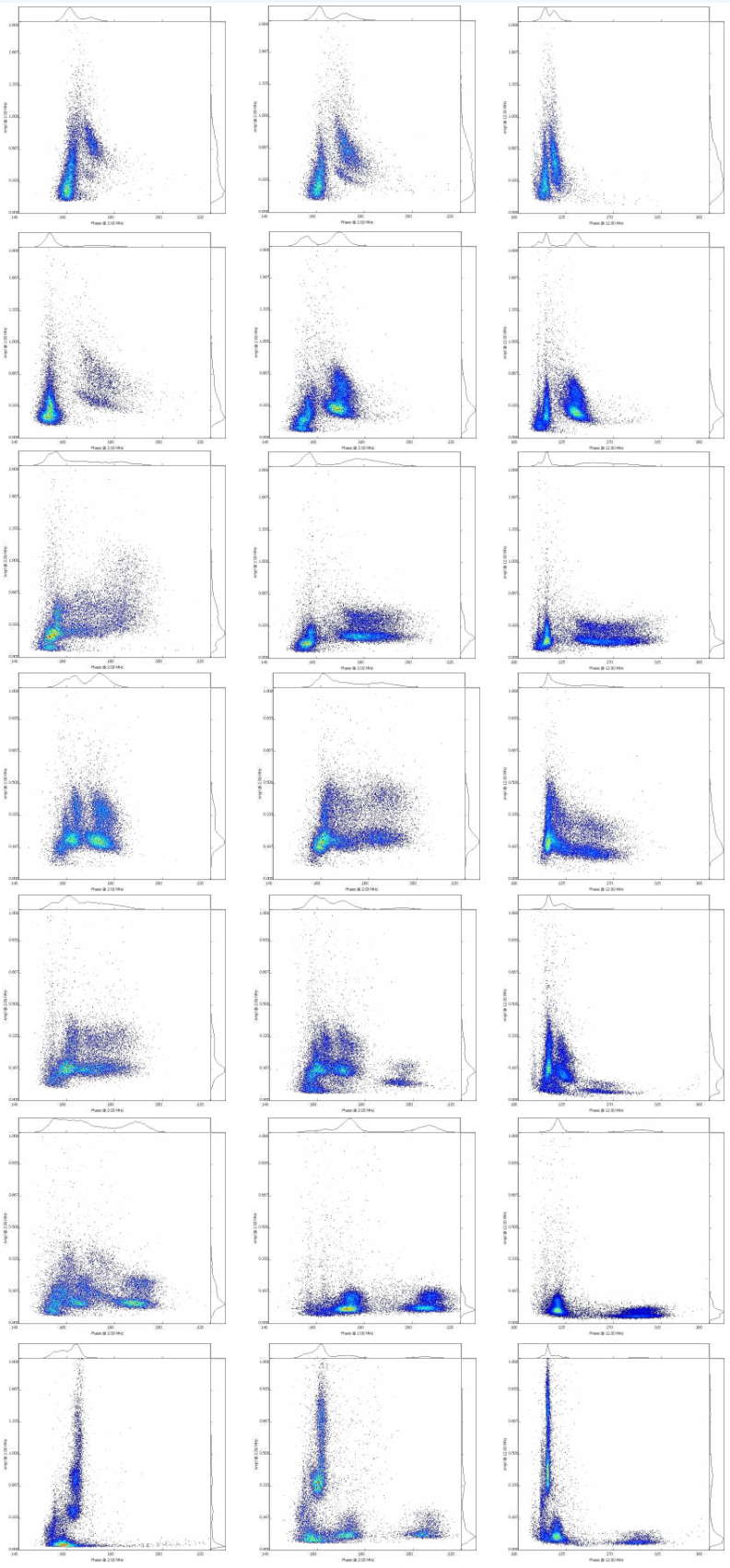
rucola - *Euruca sativa*



BROCCOLI - *BRASSICA OLERACEA ITALICA* DEVELOPMENTAL STAGES

Impedance Flow Cytometry

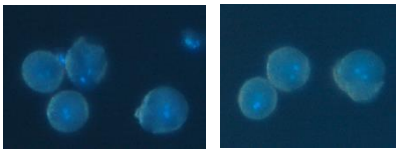
Plots of two different flowers or buds at 2 MHz and from second flower/bud at 12 MHz



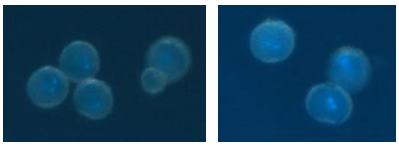
Microscopy

EtOH + HAc fixation, + DAPI staining

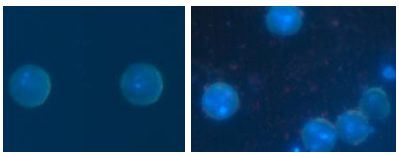
Pollen release, open flower



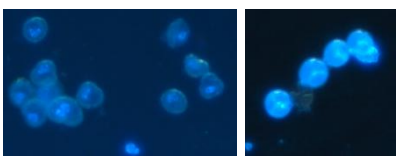
Mature pollen, closed flower



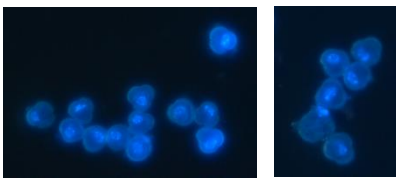
Tricellular pollen, bud 6 to 7 mm



Maturation to bicellular pollen, bud 5 mm



Late unicellular pollen, bud 4mm



Early unicellular pollen, bud 3mm

Tetrades and early unicellular pollen, bud 2mm