

Breakout Session
Hands on measuring



Plan of the session



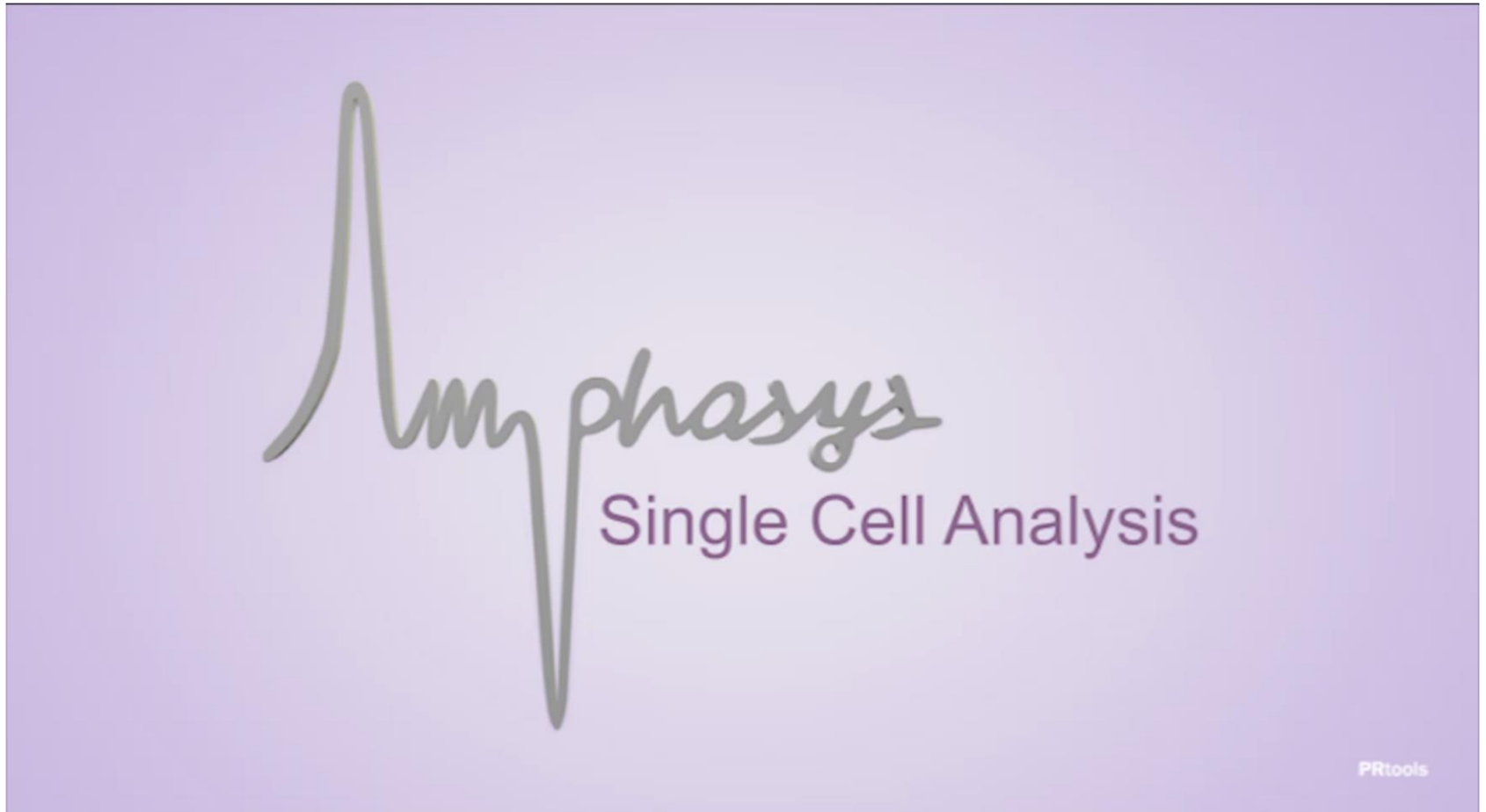
Introduction:

- How the Amphasys technology works
- The Amphasys measurements chips
- Where to find more information

Hands On Pollen Measurement:

- Introduction to the instrument
- Sample preparation (fresh and stored pollen)
- Measurements and data analysis

How we analyze pollen with IFC

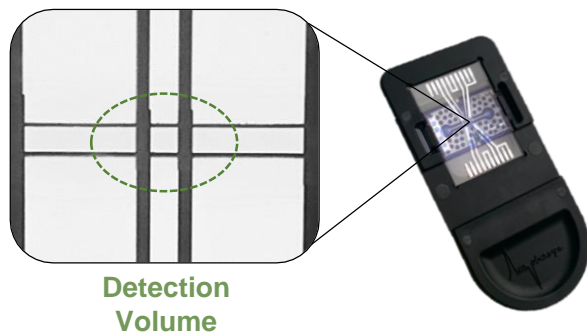


4 Chip sizes to cover over 200 species



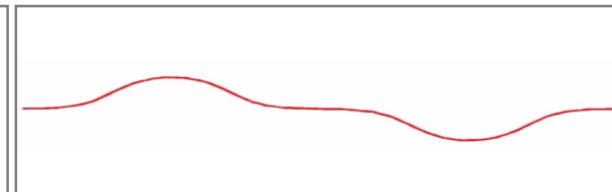
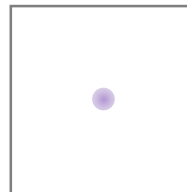
- We can analyze pollen sizes up to 200 μm
- Consult pollen analysis instructions with recommendations for buffers, filters, chips and initial settings

Chip	Pollen size	Examples
F chip	Small Pollen	Brassica
D chip	Medium Pollen	Tomato, Pepper
E chip	Large Pollen	Corn, Watermelon
G chip	Extra Large Pollen	Squash, Cotton



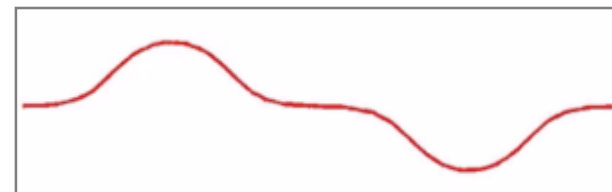
Large Channel

Weak Impedance Response



Small Channel

Strong Impedance Response



Choose the set-up for your species



Overview		General information	
Pollen size:	Pollen size can vary about 20% or even more! Polyploidy influences pollen size.		
AmphaFluid (AF) buffer recommendations:	Pollen viability: column "AF buffer pollen"		
	Microspores: column "AF buffer microspore"		
	Further purposes: also try "alternative AF buffers"		
Sample preparation:	Please refer to the Quickguides		
Dried, stored pollen:	Rehydration of pollen		
Sticky or oily pollen:	Add 0.05% Tween 20 or 80		

Species	English	Family	Class Group	Pollen-Size (µm)	Form	Ampha-Chip	Filter size	AF buffer pollen	Additional infos	AF buffer microspores	alternative AF buffers
<i>Silene vulgaris</i>	bladder campion	Caryophyllaceae	Dicots	40 - 50	round	D/E	100	6			
<i>Silybum marianum</i>	cardus marianus	Asteraceae	Dicots	70	round	E	100	6			5
<i>Sinapis arvensis</i>	charlock mustard	Brassicaceae	Dicots	25	prolate	F/D	50	7		2	2 / 5
<i>Solanum lycopersicon</i>	tomato	Solanaceae	Dicots	25	round	F/D	50	6	long stability	4	4 / 2
<i>Solanum melongena</i>	eggplant	Solanaceae	Dicots	25-30	round	D	50	6		4	4
<i>Solanum quitoense</i>	quito orange	Solanaceae	Dicots	25	round	D	50	6		6	4
<i>Solanum tuberosum</i>	potato	Solanaceae	Dicots	20	round	F/D	50	6		6	4
<i>Sonchus arvensis</i>	field milk thistle	Asteraceae	Dicots	40	round	D	100	6			5
<i>Sorghum bicolor</i>	sorghum, dari	Poaceae	Monocots	40-50	round	E	100	6		6	4
<i>Spinacia oleracea</i>	spinach	Amaranthaceae/Ch	Dicots	50	round	E	100	4		4	5
<i>Syringa vulgaris</i>	lilac	Oleaceae	Dicots	30	round	D	50	6			5
<i>Tagetes patula</i>	marigold	Asteraceae	Dicots	45	round spiny	D	100	6		5	5
<i>Taraxacum officinale</i>	dandelion	Asteraceae	Dicots	30	round spiny	D	50	6			5
<i>Theobroma cacao</i>	cacao	Malvaceae	Dicots	20	round	F	50	6		6	
<i>Thymus vulgaris</i>	thyme	Lamiaceae	Dicots	35 - 45	prolate	D	100	6			5
<i>Tilia platyphyllos</i>	largeleaf linden	Tiliaceae	Dicots	35	prolate	D	50	6			5
<i>Trifolium</i>	clover	Fabaceae	Dicots	30	round	D	50	5			6
<i>Triticum spp.</i>	wheat	Poaceae	Monocots	40 - 70	round	D/E	100	6	mature pollen short viability <0.5h	6 / 4	4
<i>Tulipa cultivars</i>	tulip	Liliaceae	Monocots	60	round	E	100	6		6	4
<i>Turnera diffusa</i>	damiana	Passifloraceae	Dicots	30	round	D	50	6			
<i>Ulmus glabra</i>	wych elm	Ulmaceae	Dicots	32	round	D	50	6			
<i>Viburnum opulus</i>	guelder-rose	Adoxaceae	Dicots	25	round	F/D	50	6			5
<i>Vicia spec</i>	vetches	Fabaceae	Dicots	30-40	round	D	50	5		5	
<i>Vicia faba</i>	broad bean	Fabaceae	Dicots	50	prolate	D/E	100	5		5	
<i>Vicia villosa</i>	winter vetch	Fabaceae	Dicots	35	prolate	D	50	5			6
<i>Vinca spec.</i>	vinca	Apocynaceae	Dicots	60-80	round	E	100	5			6
<i>Viola cornuta</i>	violet	Violaceae	Dicots	40	round	D/E	100	6			
<i>Viola wittrockiana</i>	violet	Violaceae	Dicots	70	round	E	100	6			
<i>Vitis vinifera</i>	grape	Vitaceae	Dicots	30	round	F/D	50	6			
<i>Wisteria sinensis</i>	glycine	Fabaceae	Dicots	30	triangular	D	50	6			5
<i>Zantedeschia aethiopica</i>	calla	Araceae	Monocots	50	round	E	100	6			
<i>Zea mays</i>	corn, maize	Poaceae	Monocots	80 - 120	round	E	150	6	mature pollen 2 - 4h viability	6	

Amphasys Downloads – Reference Material



- Pollen Analyzer ▾
- Tec Pollen Analyzer ▾
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- Pollen Downloads
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Product Downloads

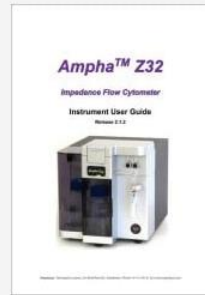
Ampha Z32
Brochure



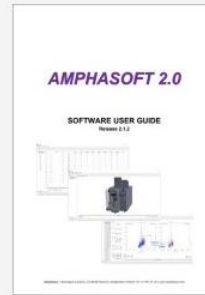
Autosampler
Flyer



Ampha Z32
User Guide



AmphaSoft
User Guide



Battery
User Guide



Impedance Flow
Cytometry



Pollen Analysis
Instructions



Amphasys
Product List



Let's start measuring pollen viability!

- I. Rehydration of stored pollen
- II. Sample preparation and measurement of fresh pollen
- III. Measurements of stored pollen



Results: What do we see in a scatterplot?



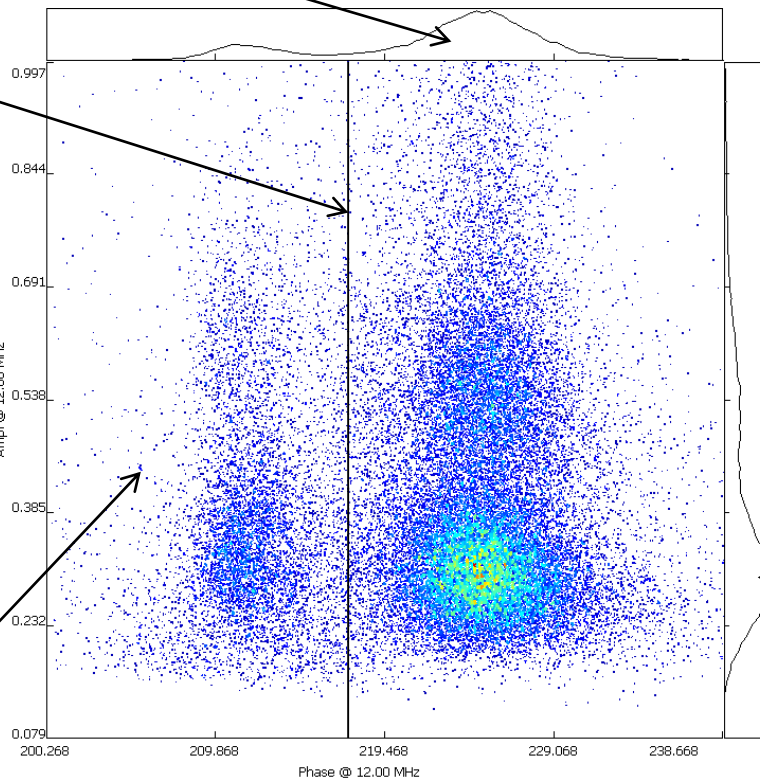
Phase Histogram

Vertical Gate

Amplitude

Amplitude Histogram

Each measured pollen is one dot in the plot



Phase

Dead cells

Viable cells