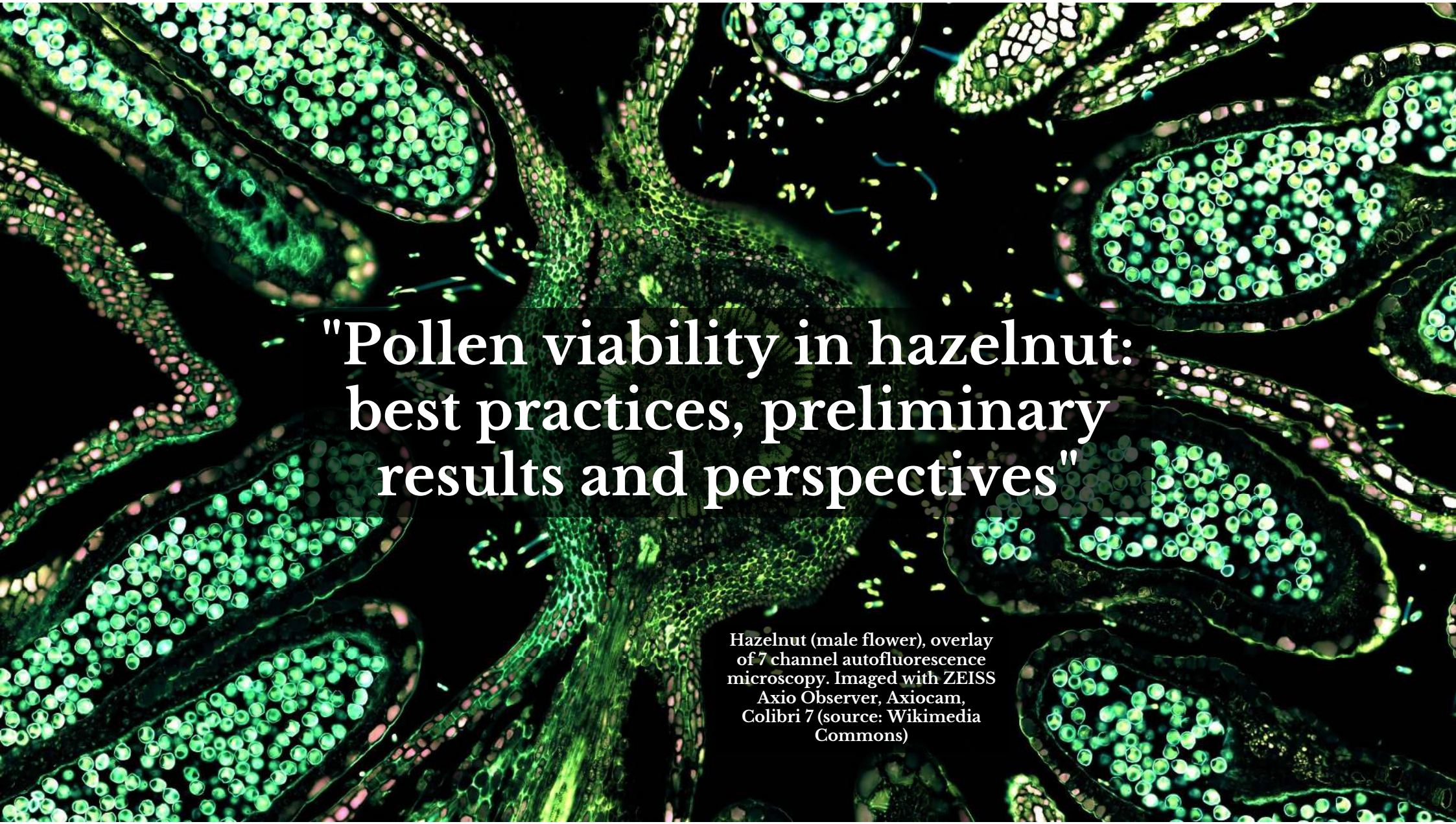
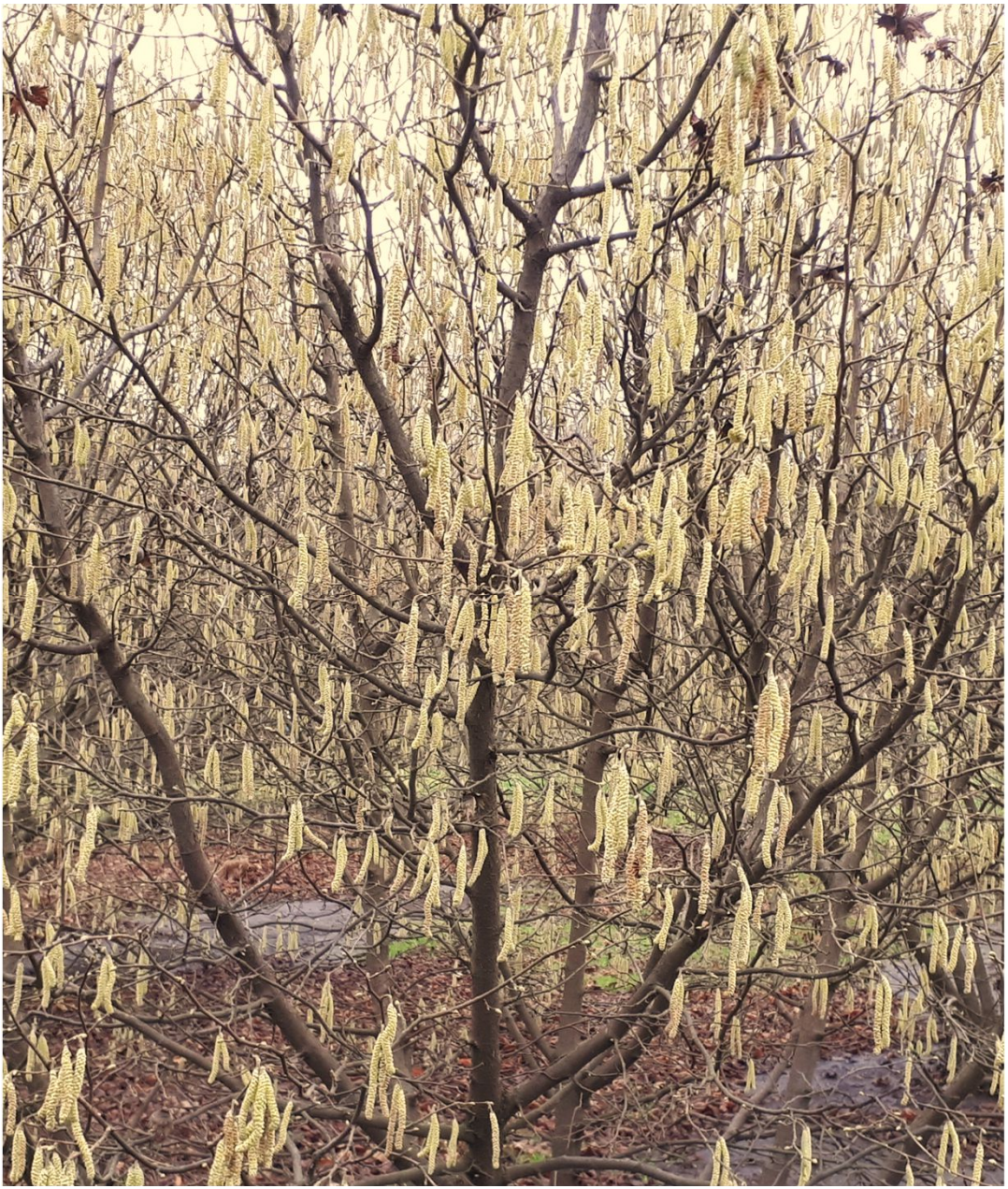


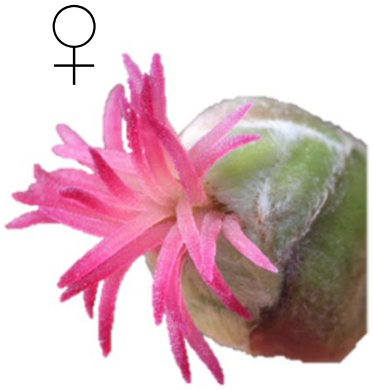
"Pollen viability in hazelnut: best practices, preliminary results and perspectives"

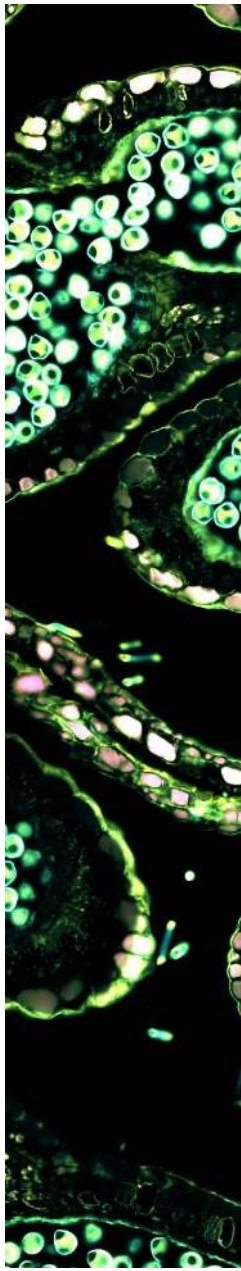
Hazelnut (male flower), overlay of 7 channel autofluorescence microscopy. Imaged with ZEISS Axio Observer, Axiocam, Colibri 7 (source: Wikimedia Commons)

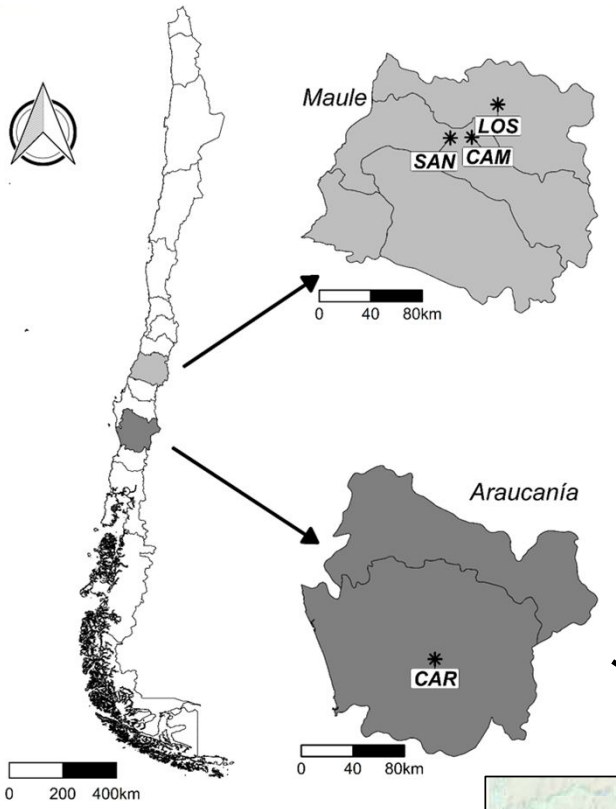


Corylus avellana reproduction:

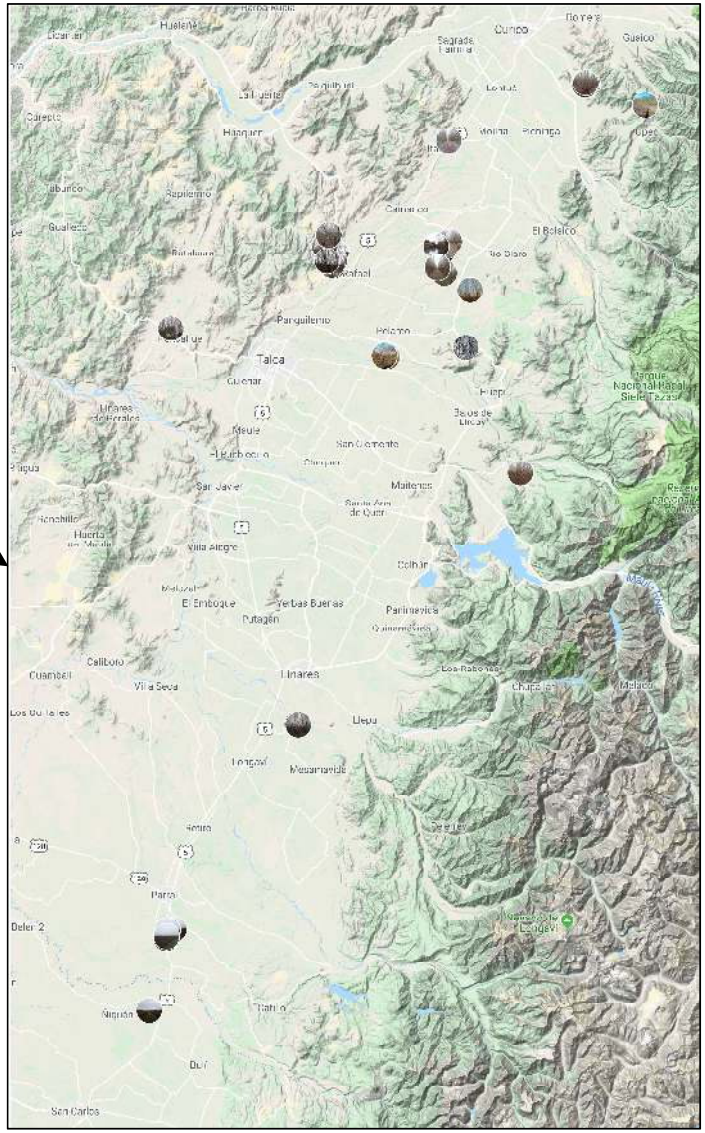
- **Anemophilous** species (wind pollinated)
- **Mid-winter flowering**
- **Monoecious**
- **Obligate out-crosser**, sporophytic self-incompatibility
- **Dry stigmas**
- **Frequent pollen sterility** especially among cultivated variaties

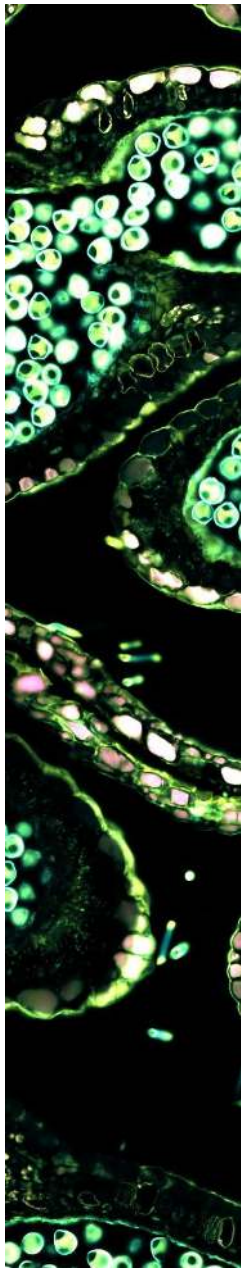


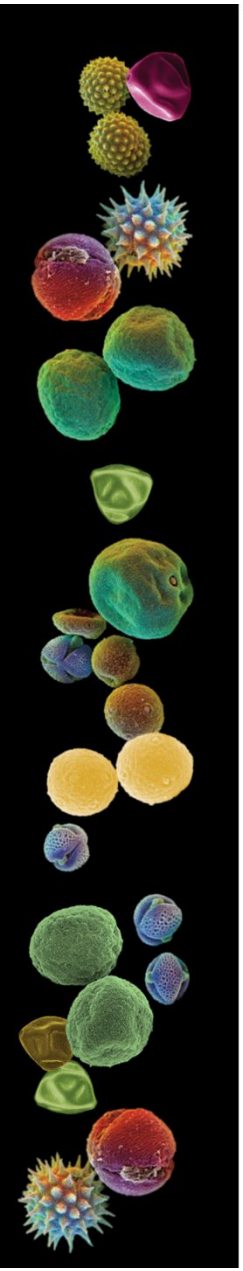




Chilean sampling campaign



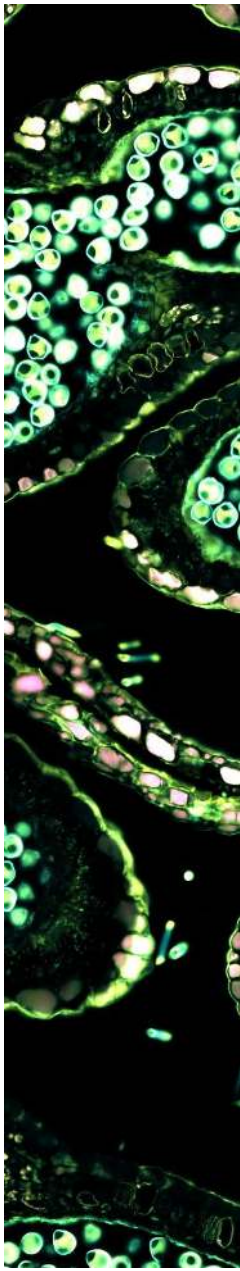




Today's topics

1. Pollen hydration dynamics revealed by Impedance Flow Cytometry: how hazelnut pollen viability changes during re-hydration and the differences between samples and varieties.

2. Validation of AmphaZ32 results through the use of image analysis: how quantitative microscopy could be used to validate your viability data and to search for new and unexpected pollen populations

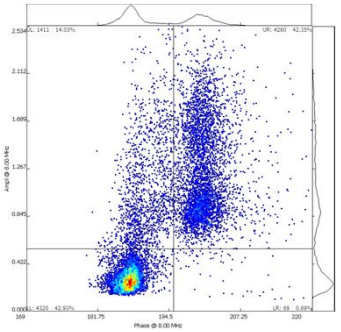


1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

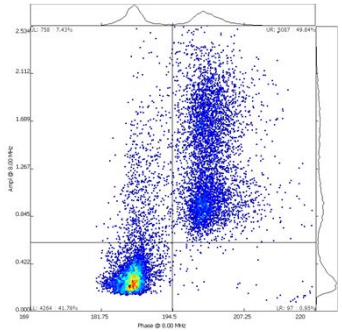
Experimental setup:

- **5 different pollen samples** from 5 hazelnut varieties
- **One replica** for each sample type
- Progressive **hydration inside improvised humid chambers** with RH around 100 %
- Impedance analysis with AmaphaZ32 at each 30' time-steps from T0 to T420'
- Buffer AF6, chip 120 µm

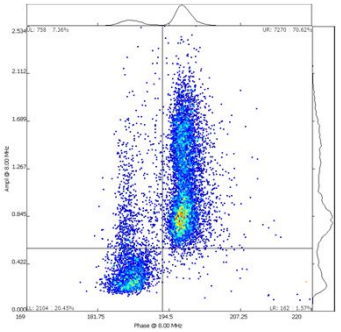
Sample 1



Sample 2



Sample 3



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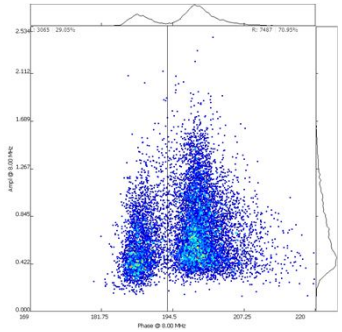
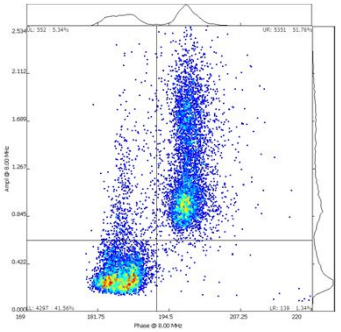
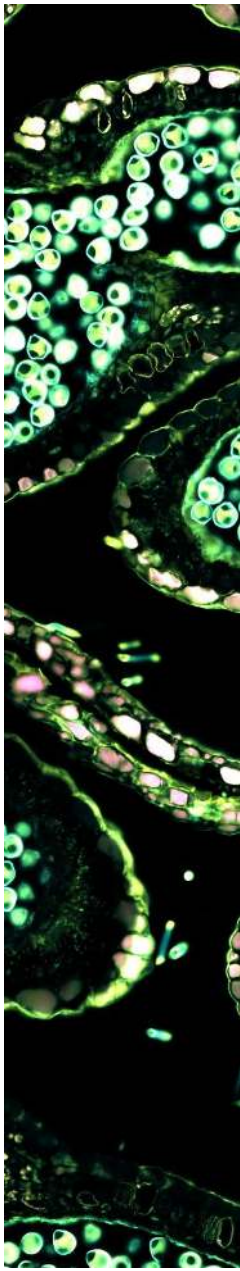


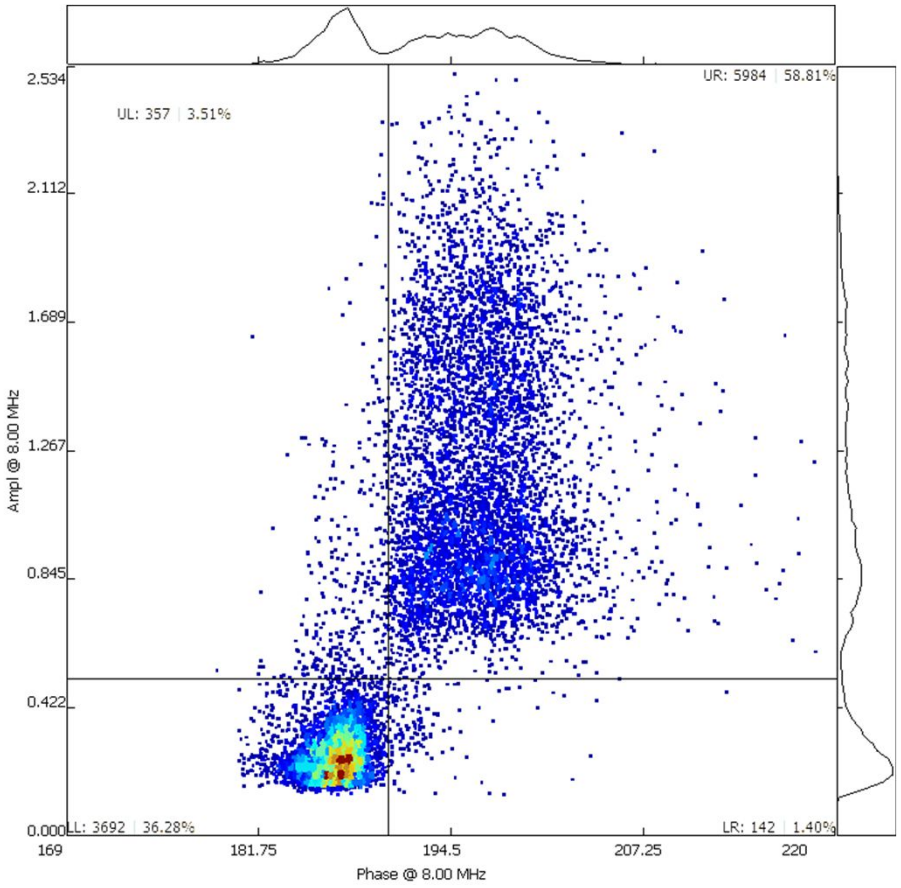
Sample 5

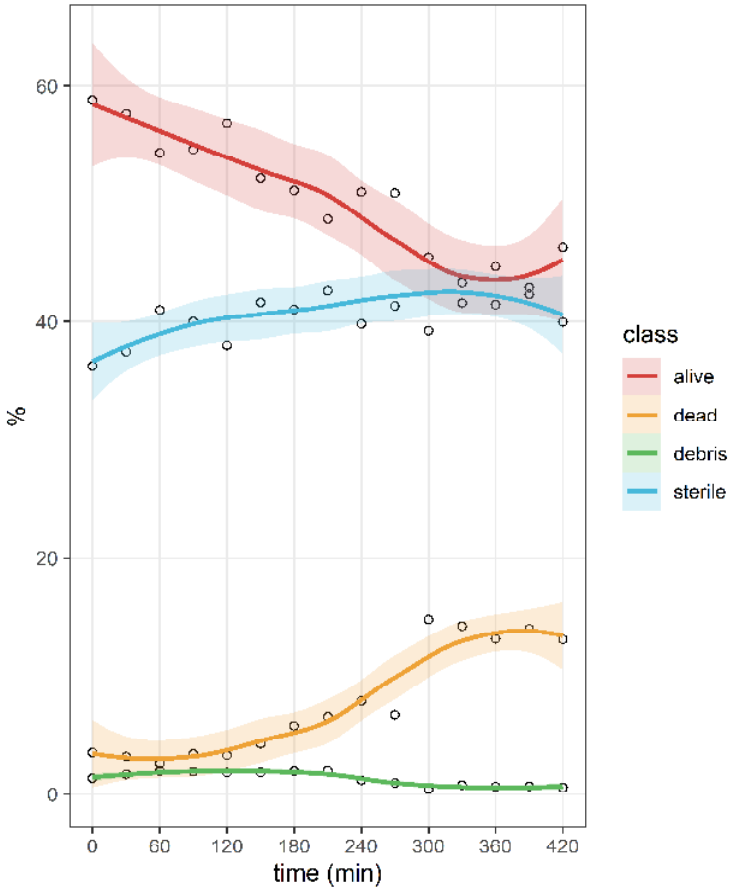


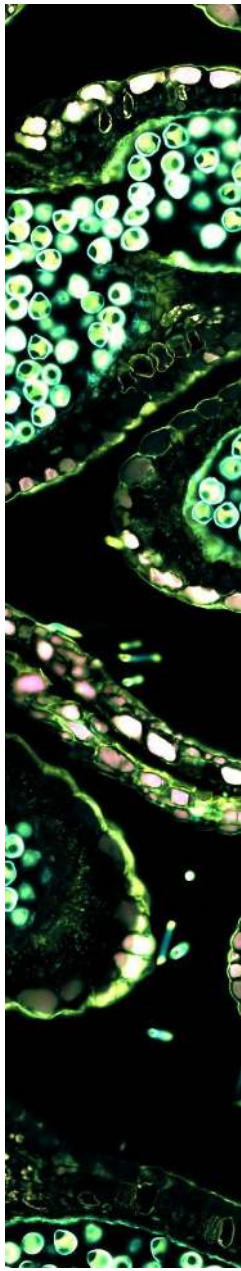


1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Sample 1

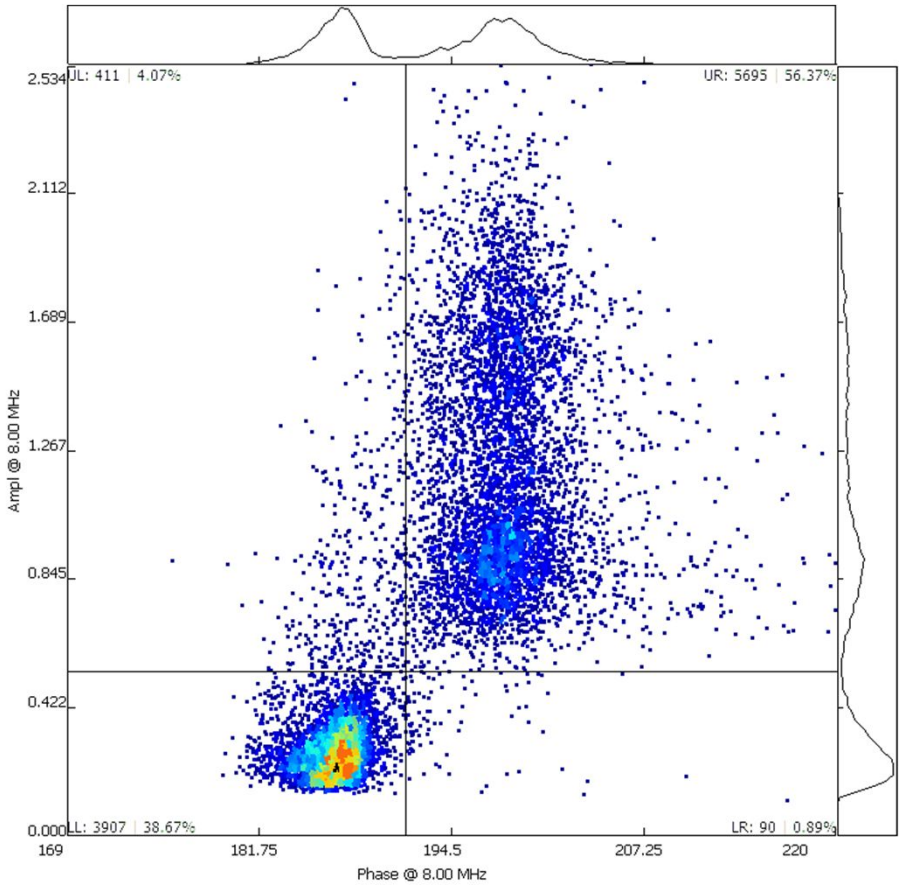


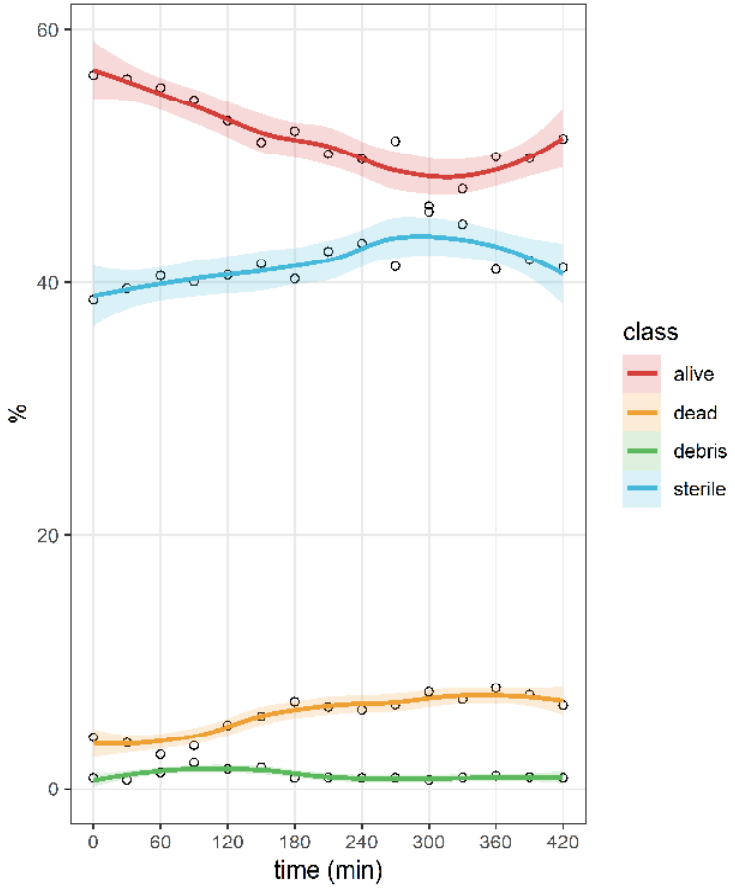


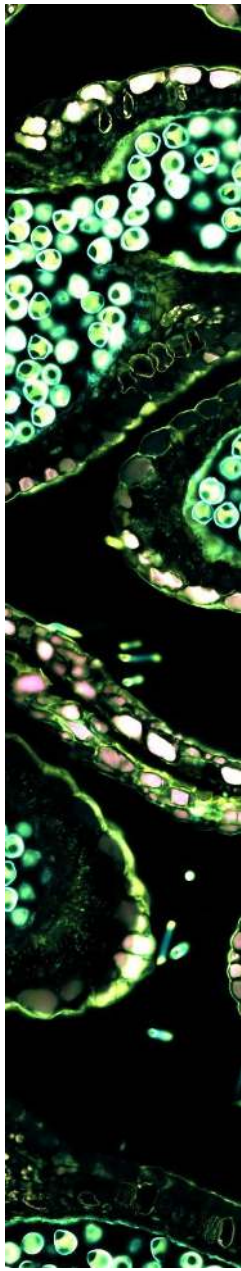


1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Sample 2

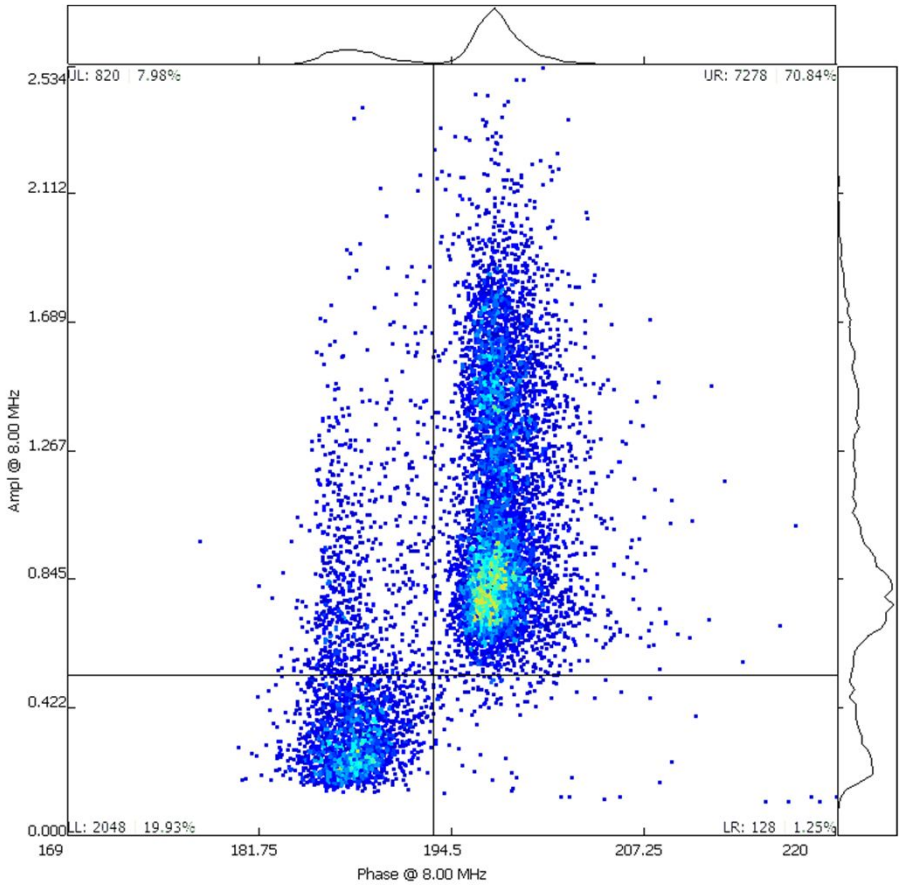


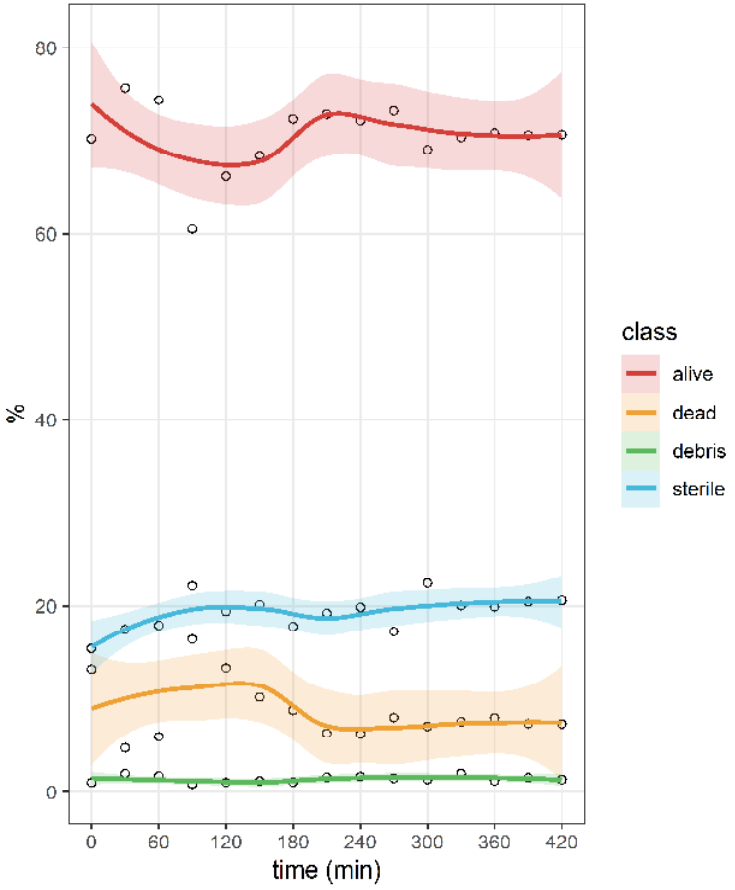


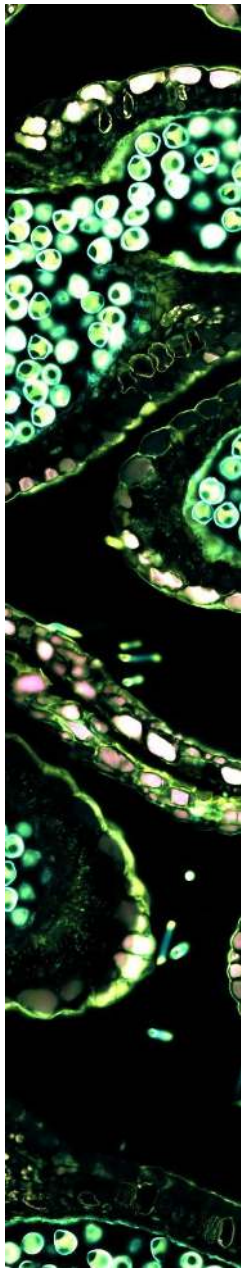


1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Sample 3

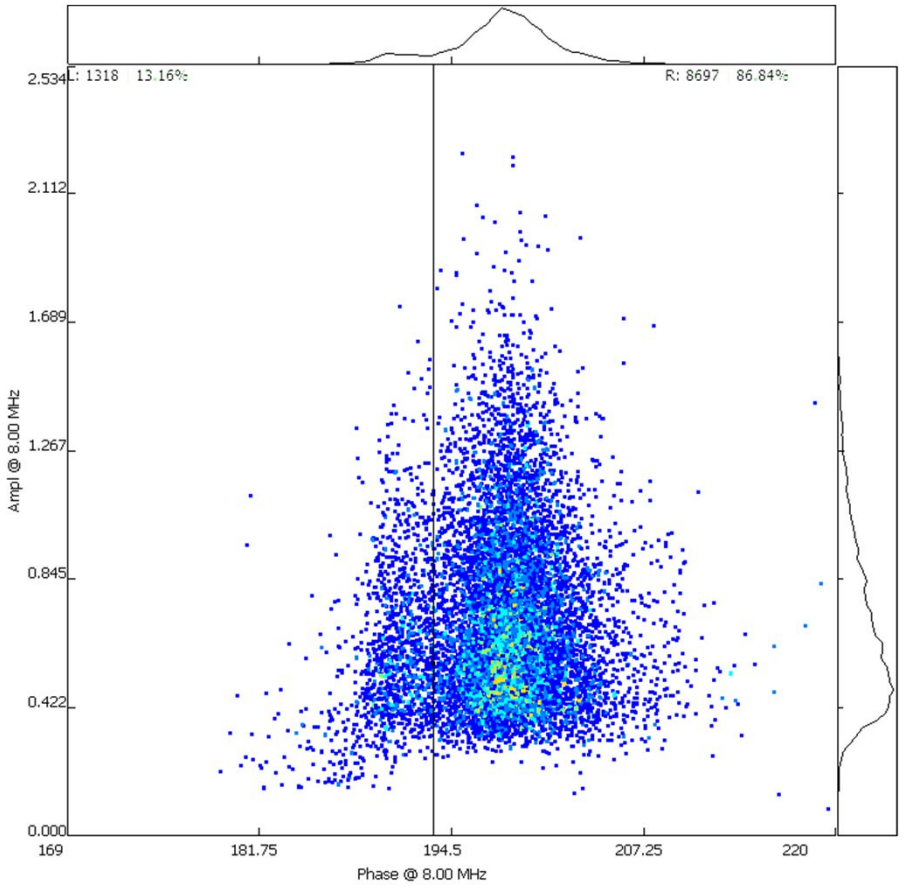


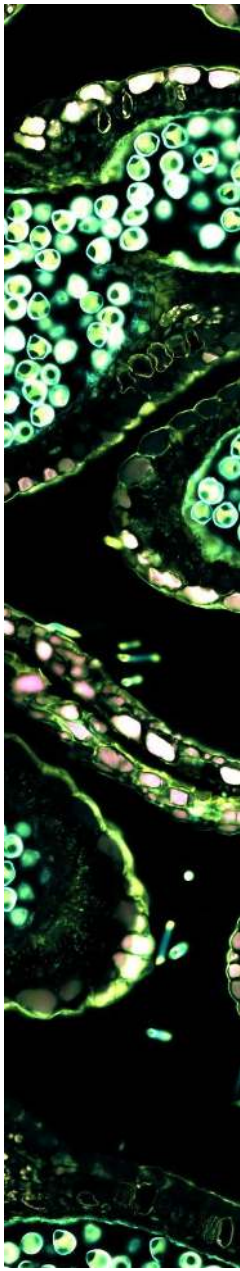




1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

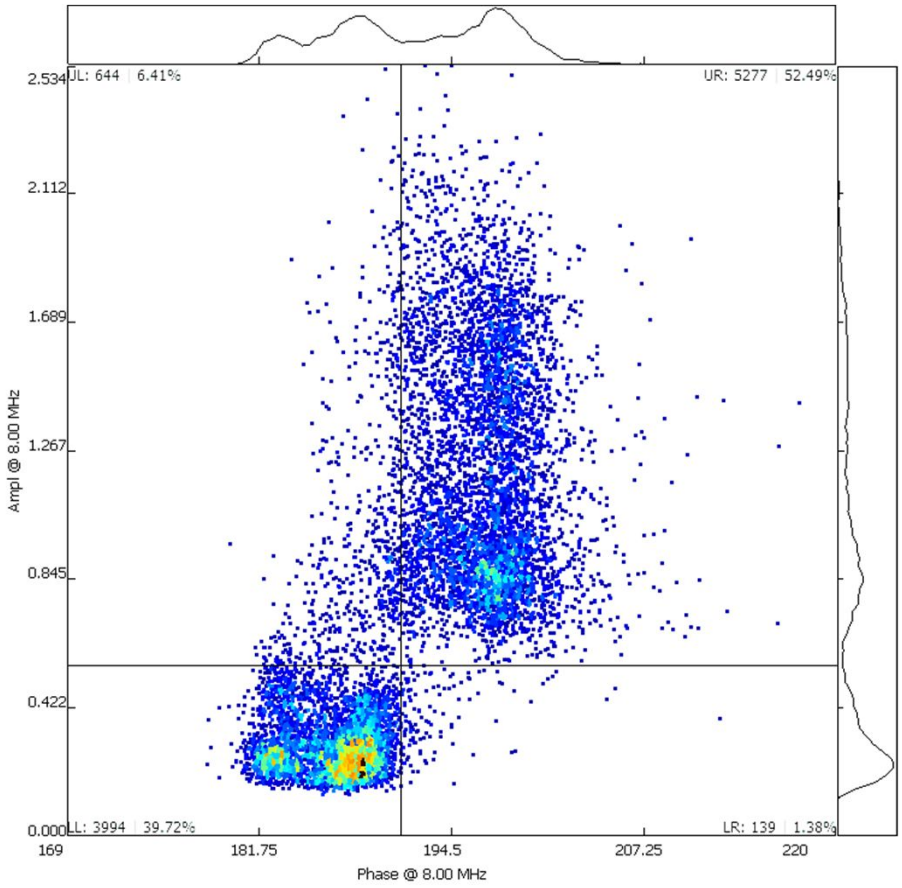
Sample 4

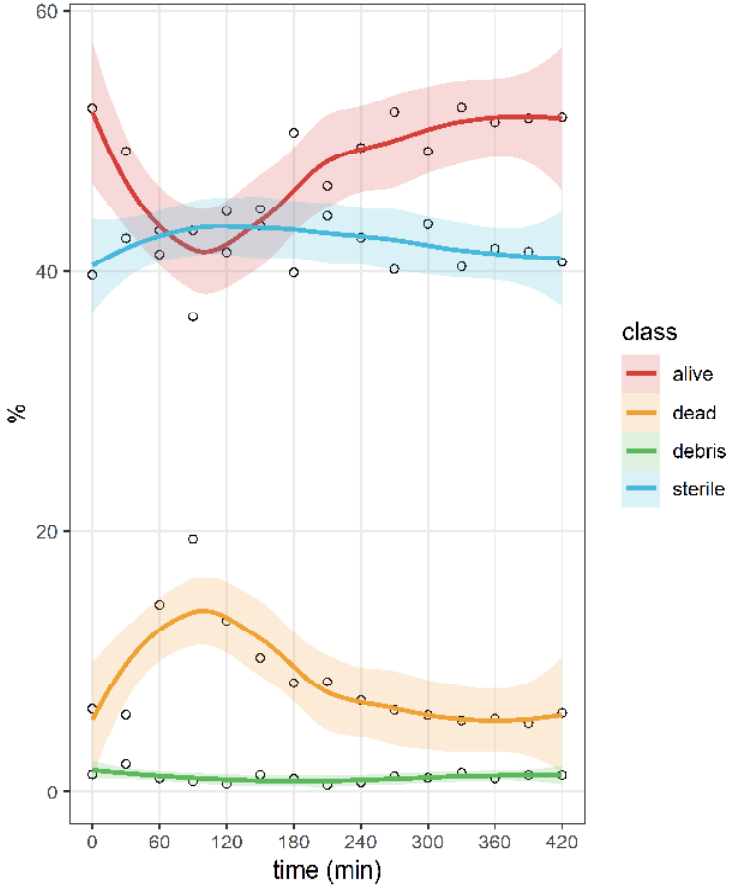


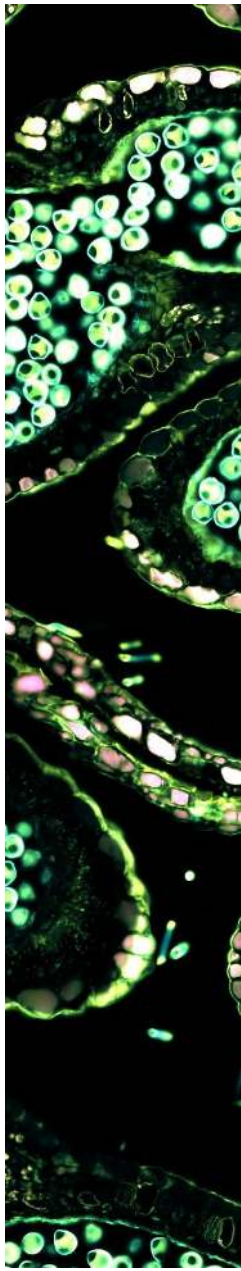


1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Sample 5

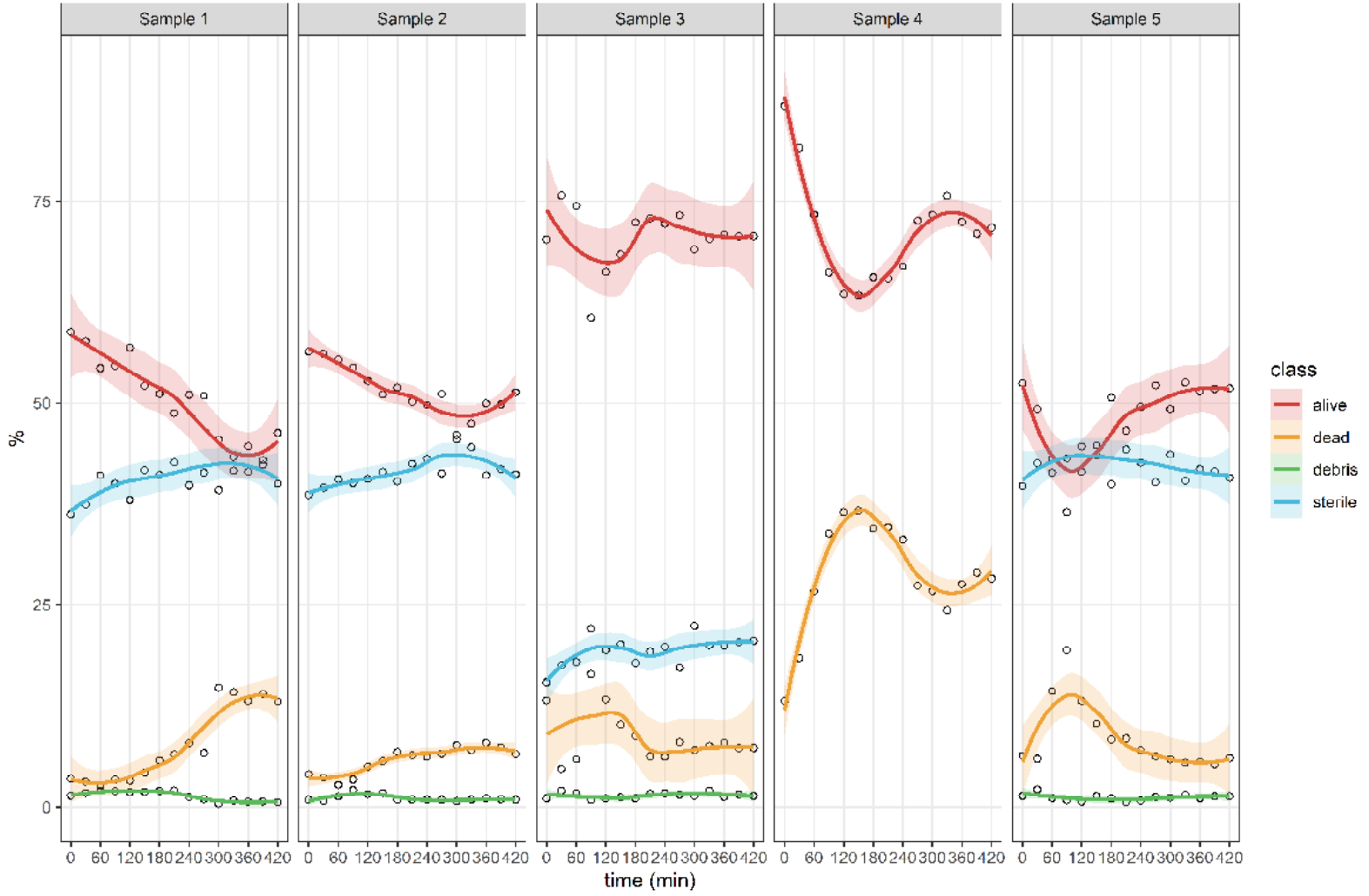


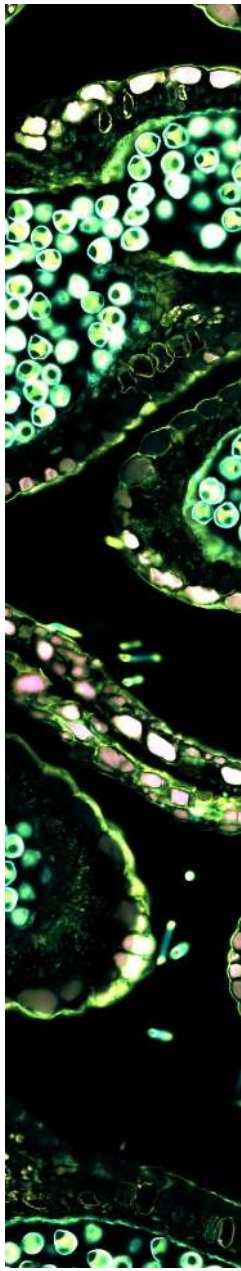




1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

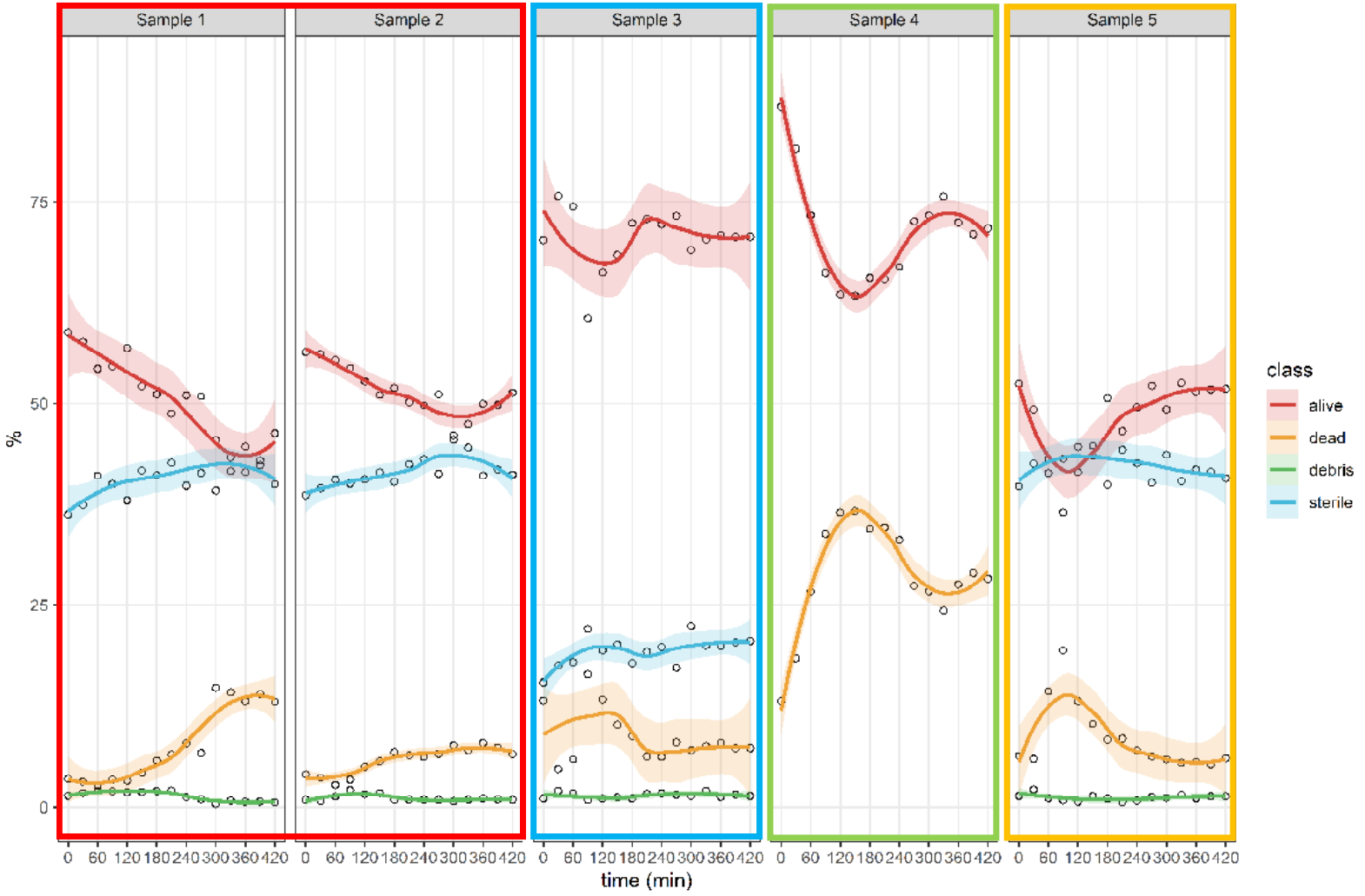
Final comparison

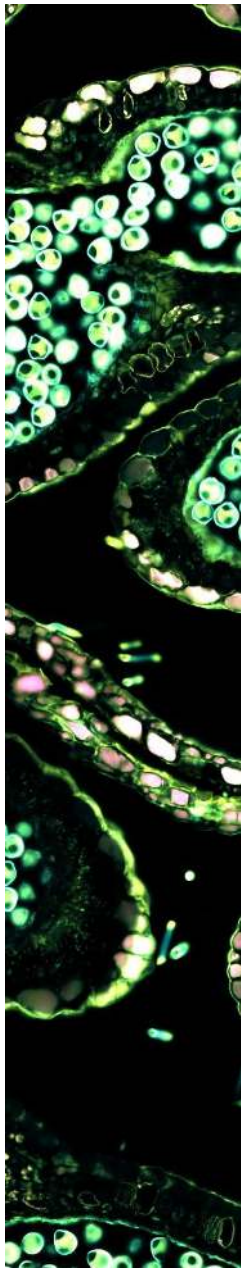




1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

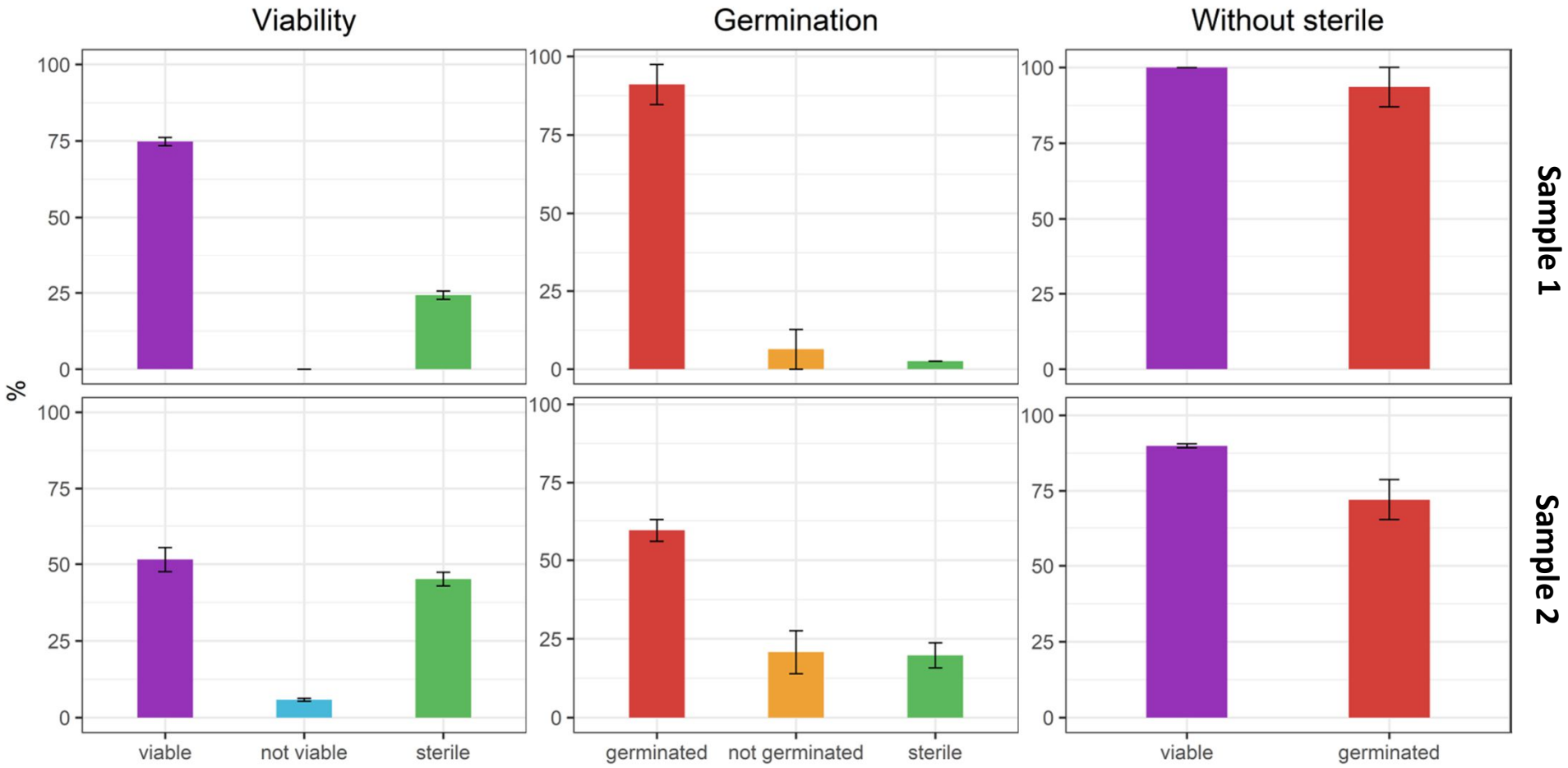
Final comparison

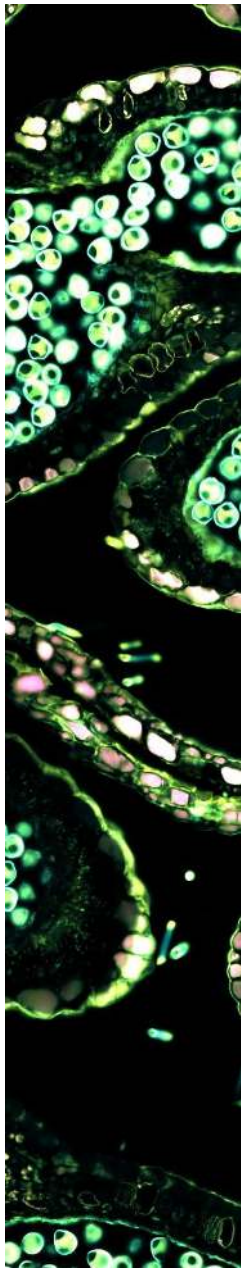




1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Prolonged pollen hydration and proper medium composition are essential to obtain high germination rates in hazelnut pollen





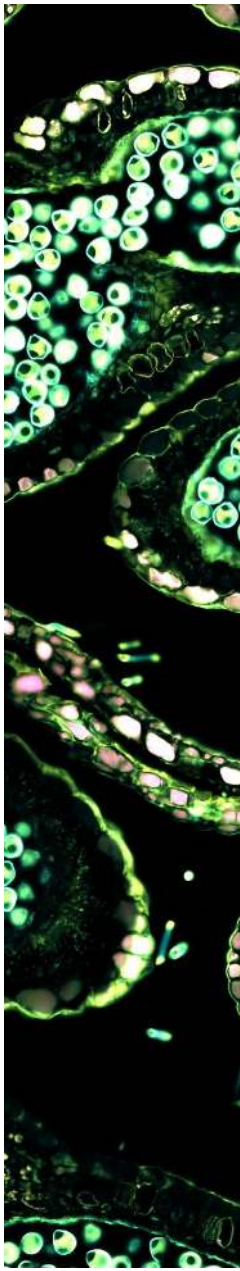
1. Pollen hydration dynamics revealed by Impedance Flow Cytometry

Conclusions

- Long hydration times are useful for a clearer separation between dead/alive pollen populations
- Sample/cultivar-specific behaviour
- Appropriate germination medium and prologed hydration are essential to obtain satisfying pollen germination rates in an anemophylous species with long-lived pollen and dry stigmas

Perspectives

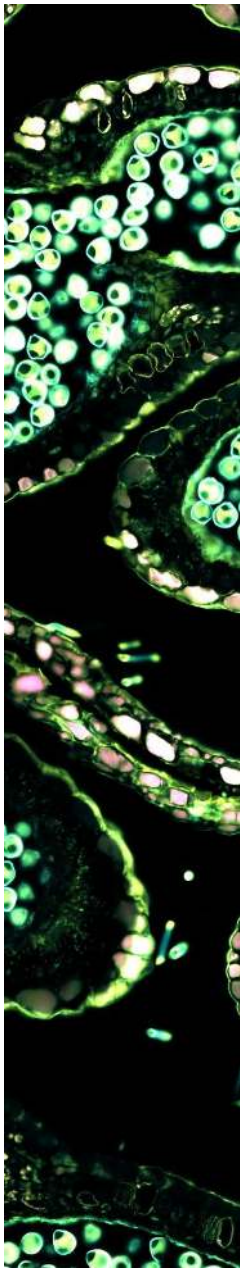
Repeat the experiment on multiple replicas/sample and for, possibly, even longer time-periods



2. Validation of AmphaZ32 results through the use of image analysis

Experimental setup:

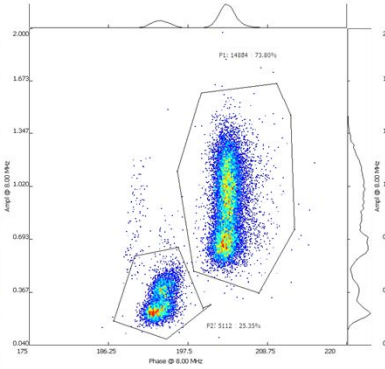
- **viability increments** by 25 %, from 0 % (all dead) to 100 % (all alive) using samples of one hazelnut cultivar
- **Three replicas** for each viability increment
- Sample analysis with **AmphaZ32** (chip 80 µm)
  - ❖ analysis time/sample = **seconds**
  - ❖ measured pollen grains/replica = **20.000**
- Sample analysis with **dye-eclusion technique in bright-field microscopy** (custom method)
- Automated image analysis with an open-source software
  - ➢ Analysis time/sample = **many hours**
  - ➢ measured pollen grains/replica = **around 5000**
- Same buffer AF6 for both impedance measurements and staining procedure for a better apple-to-apple comparison



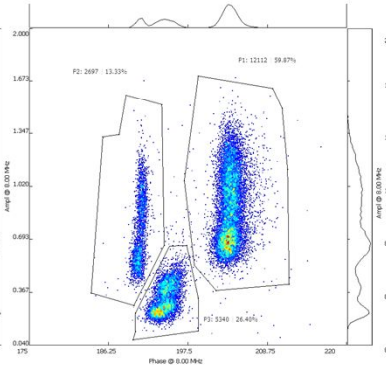
2. Validation of AmphaZ32 results through the use of image analysis

AmphaZ32

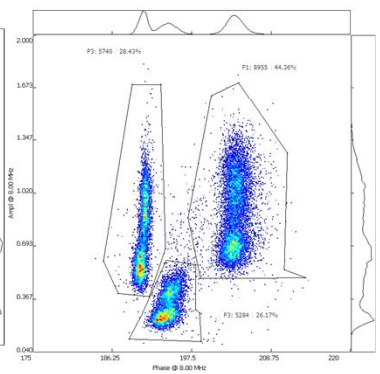
100 % viable



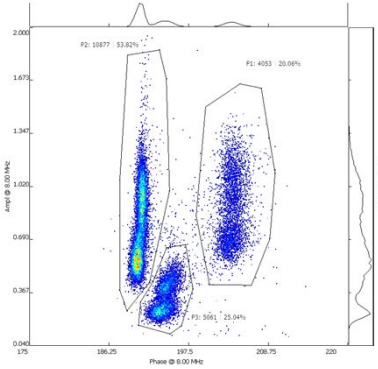
75 % viable



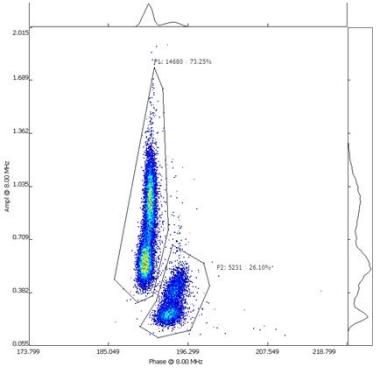
50 % viable

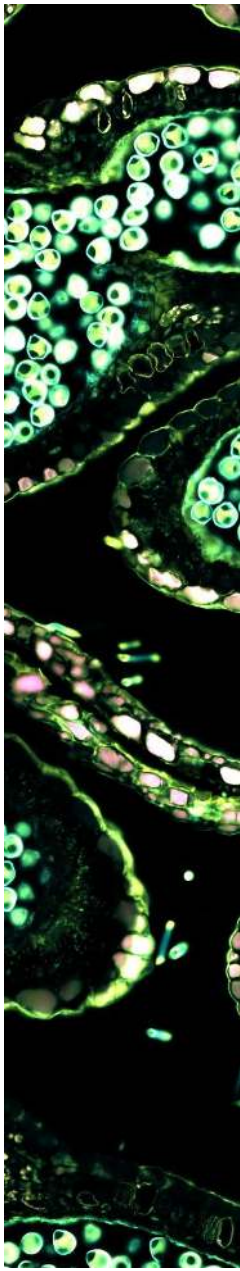


25 % viable



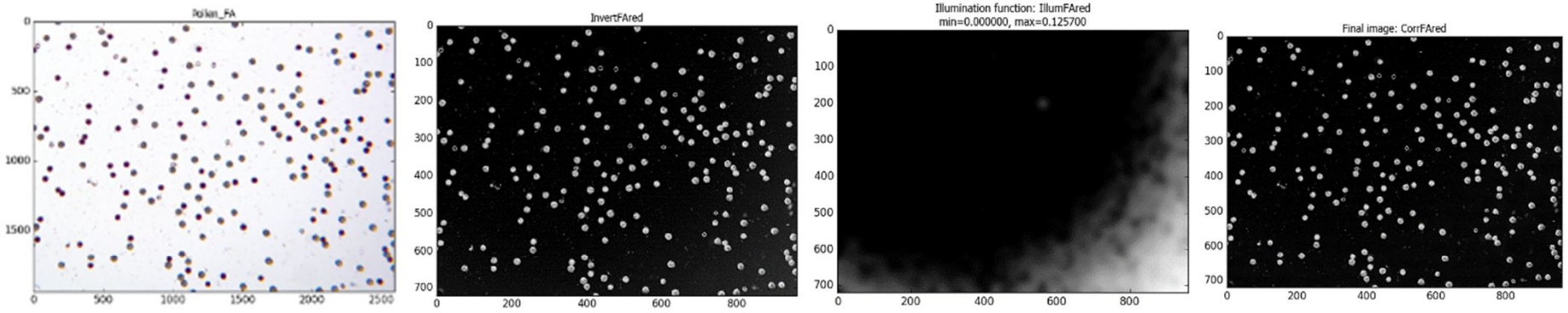
0 % viable

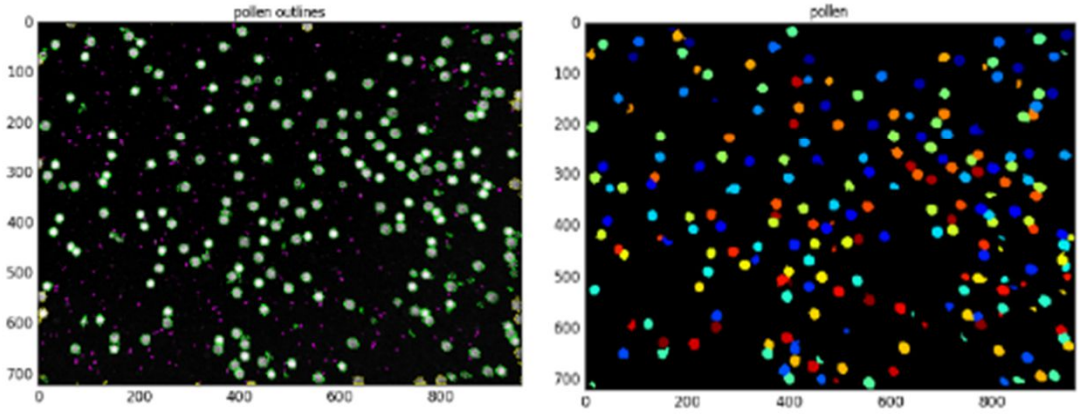




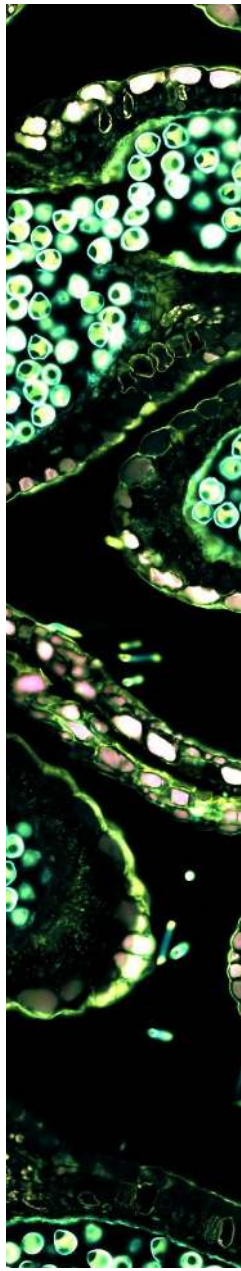
2. Validation of AmphaZ32 results through the use of image analysis

Image Analysis



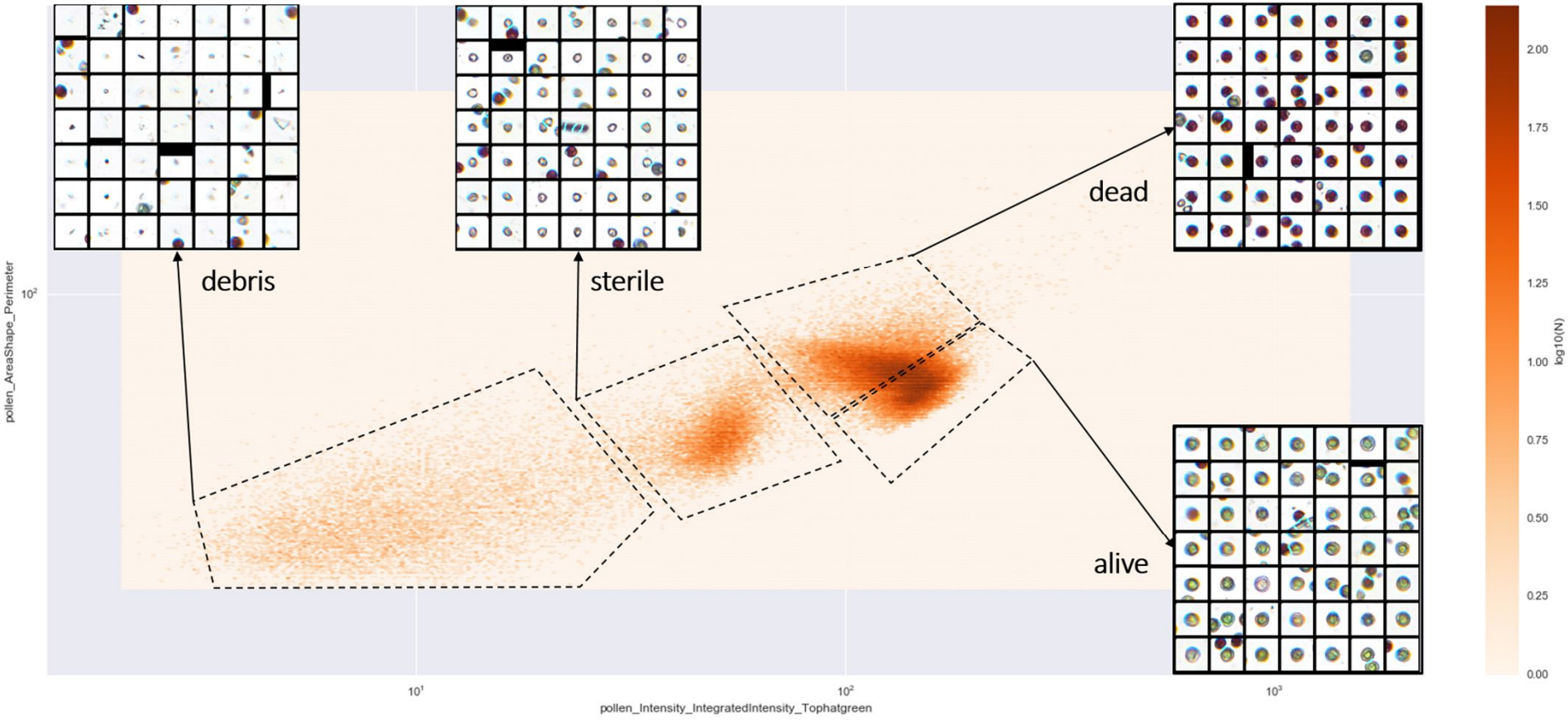


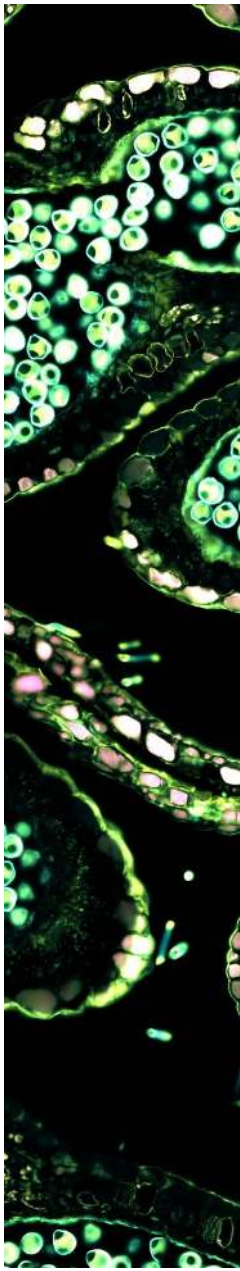
	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM
4587	65.049	45.256	22.679	66	0	88	135.274	124.329	135.368
4588	41.354	11.724	8.431	7	0	37	168.450	125.704	168.227
4589	68.960	44.515	24.174	0	0	85	177.161	128.714	177.497
4590	68.849	54.375	21.671	77	8	88	199.535	129.014	199.511
4591	65.384	47.569	23.038	66	0	89	24.340	131.987	24.304
4592	47.278	30.028	16.239	33	0	62	32.755	132.783	32.601
4593	62.031	40.303	20.843	52	0	78	101.657	133.277	101.616
4594	56.107	36.841	20.086	63	0	69	122.781	134.184	122.859
4595	65.384	70.549	24.023	95	13	127	165.230	137.821	165.303
4596	60.578	55.672	20.253	70	11	92	141.911	142.388	141.899
4597	56.554	35.903	23.266	6	0	76	13.262	144.921	13.348
4598	62.701	37.749	18.499	47	0	72	117.871	145.692	117.955
4599	52.866	34.300	18.901	57	0	64	199.439	144.906	199.440
4600	62.143	55.401	24.071	82	0	93	177.194	145.484	177.339
4601	107.855	182.845	18.232	185	61	204	163.491	147.647	163.507
4602	51.860	27.899	15.640	46	0	50	38.062	146.884	38.145
4603	64.490	37.279	20.095	66	0	71	80.894	147.810	80.872
4604	57.113	45.847	24.797	70	0	92	69.580	151.774	69.806
4605	42.695	2.369	2.049	0	0	9	111.538	153.791	111.832
4606	68.402	42.382	21.582	68	2	82	12.383	155.705	12.368
4607	25.259	34.531	12.588	49	4	53	199.008	153.809	199.071
4608	54.207	54.825	25.725	77	0	100	121.912	155.162	121.882



2. Validation of AmphaZ32 results through the use of image analysis

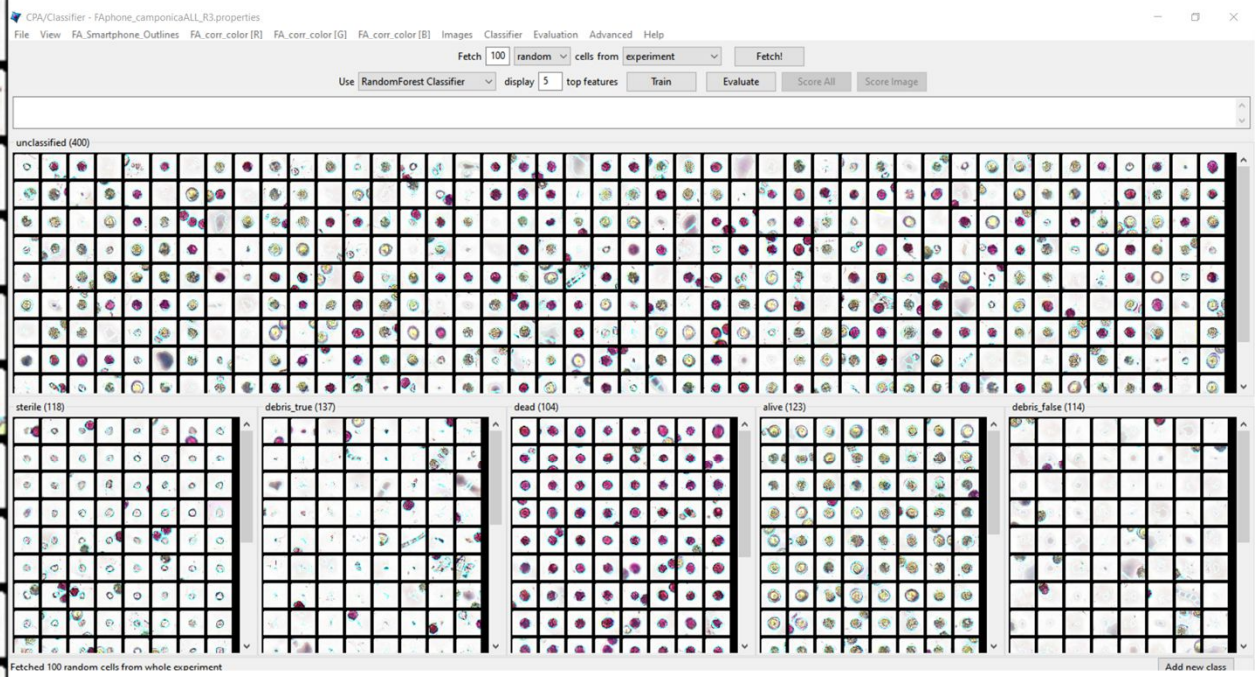
Data exploration by fetching pollen grains from density plots

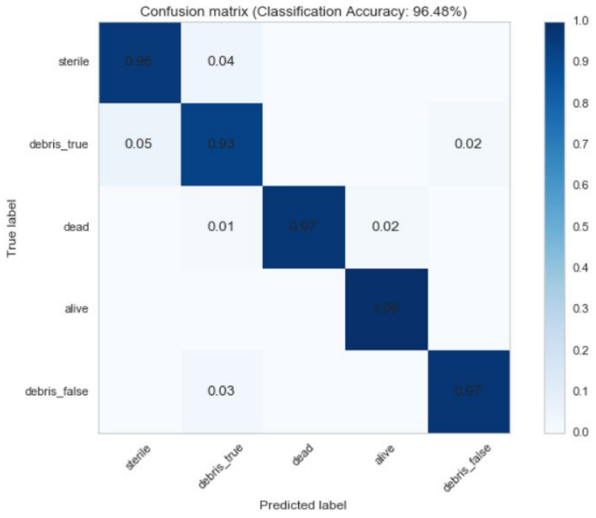


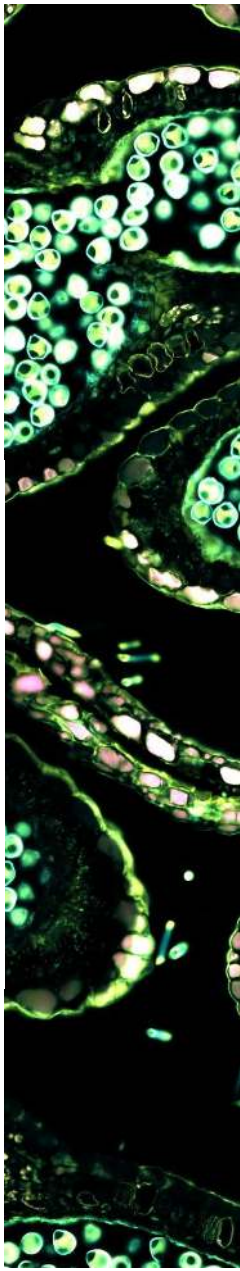


2. Validation of AmphaZ32 results through the use of image analysis

Supervised classification with machine learning algorithms



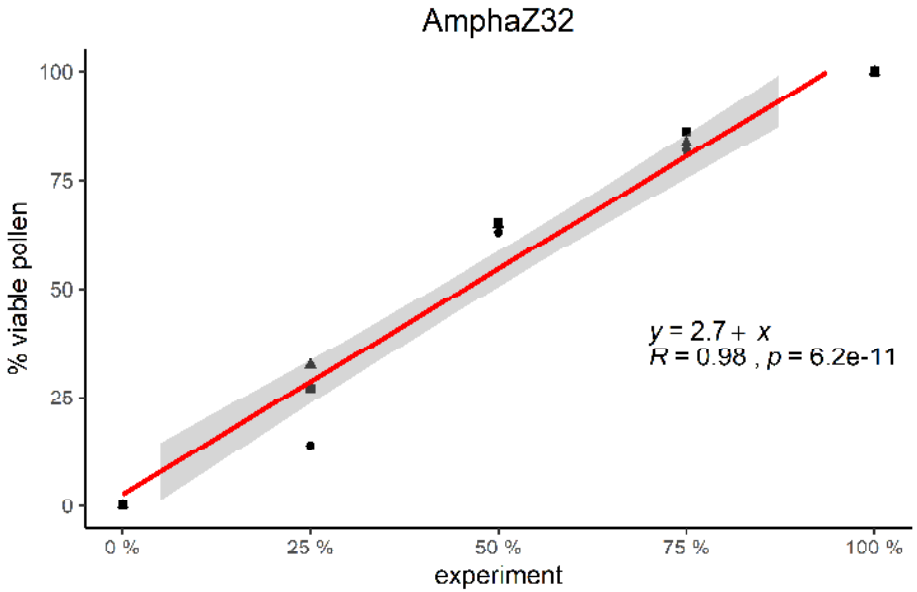


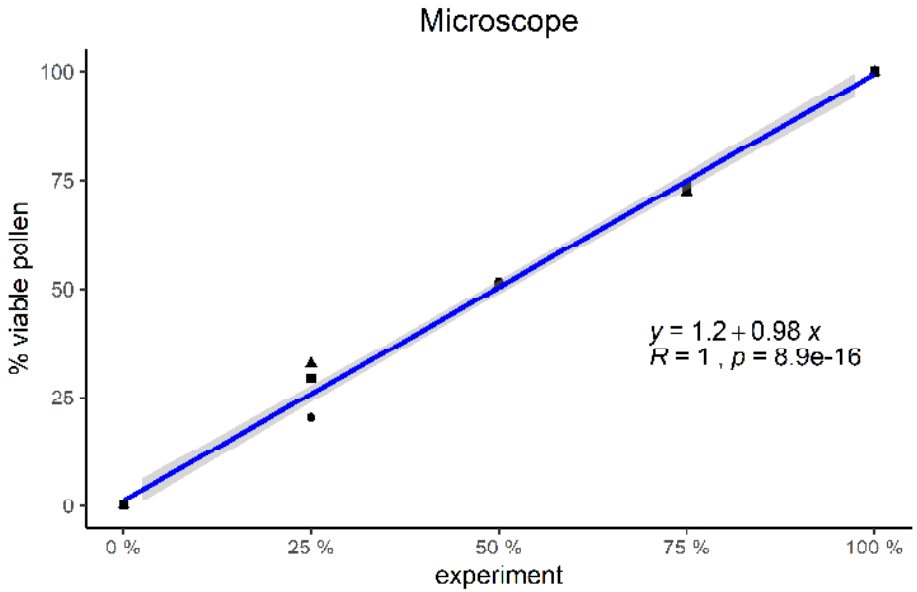


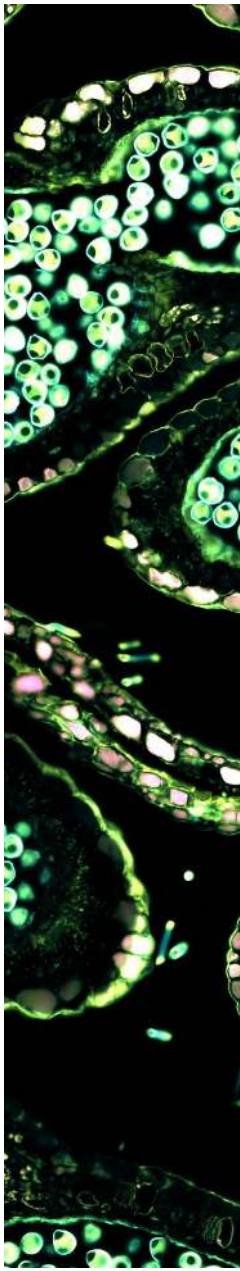
2. Validation of AmphaZ32 results through the use of image analysis

Results

Only viable cells considered



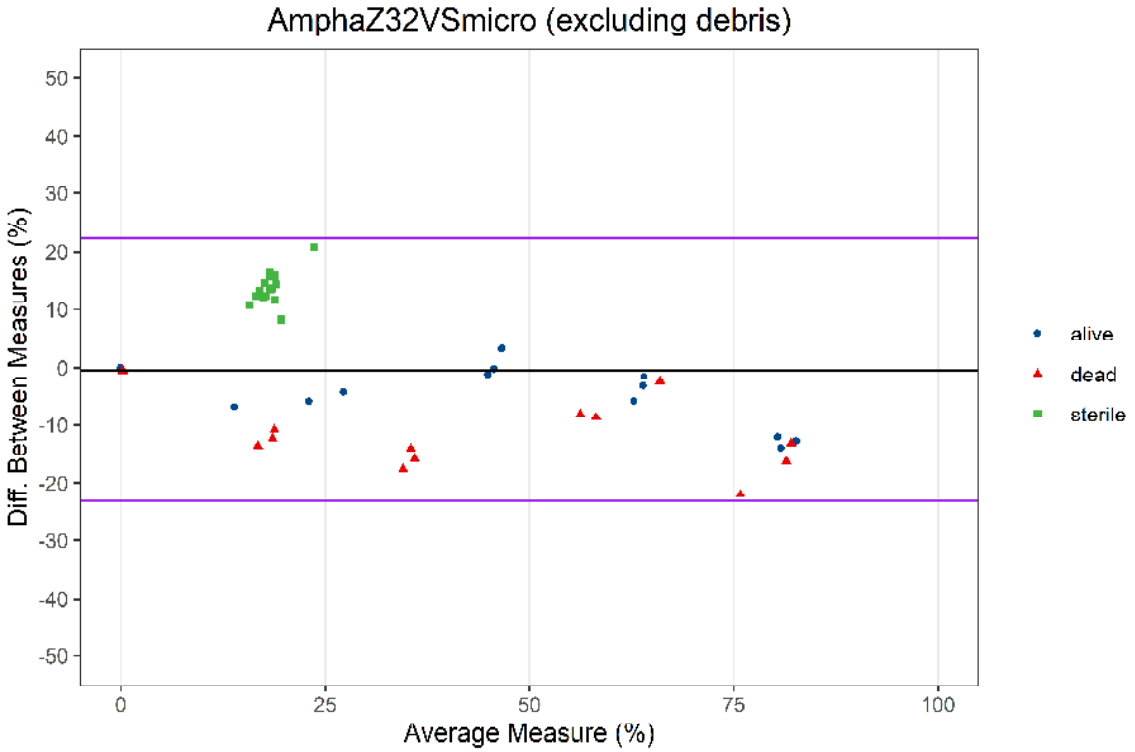


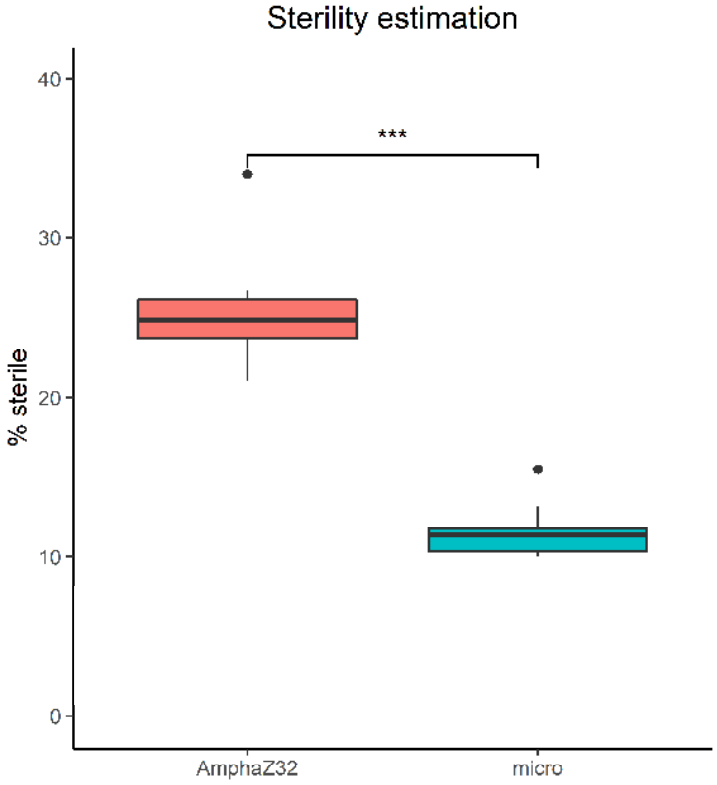


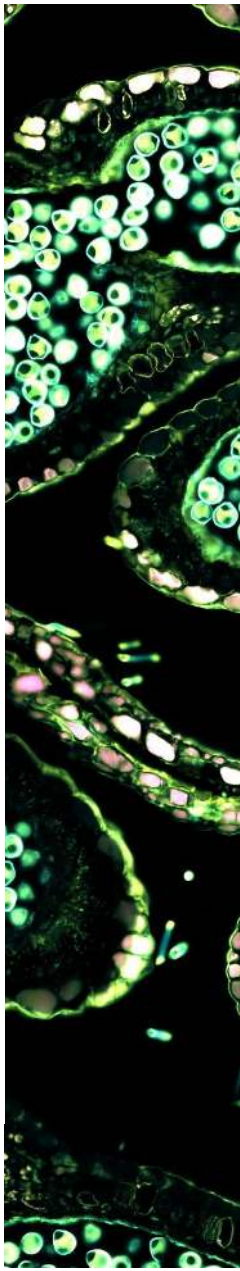
2. Validation of AmphaZ32 results through the use of image analysis

Results

Overall comparison (viable, dead and sterile)



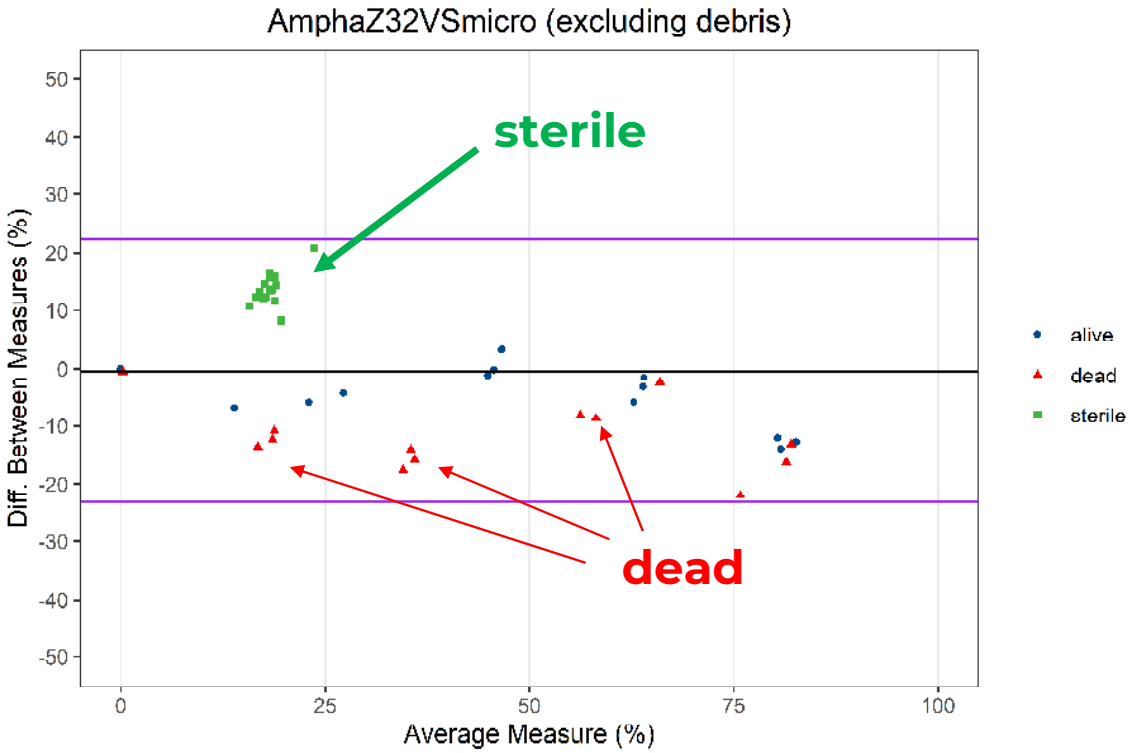


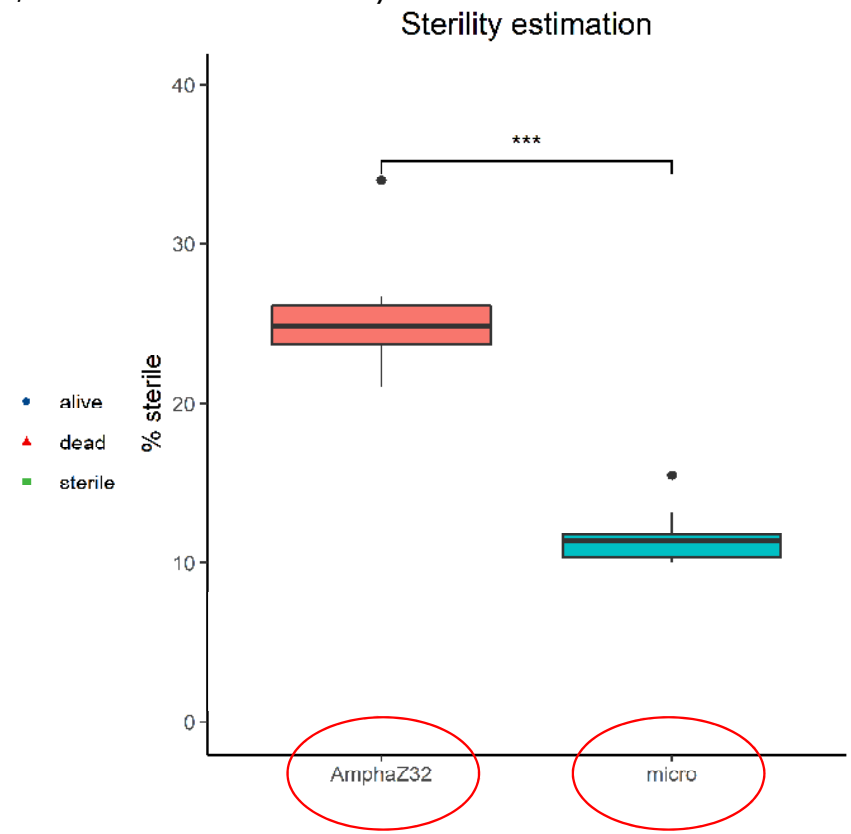


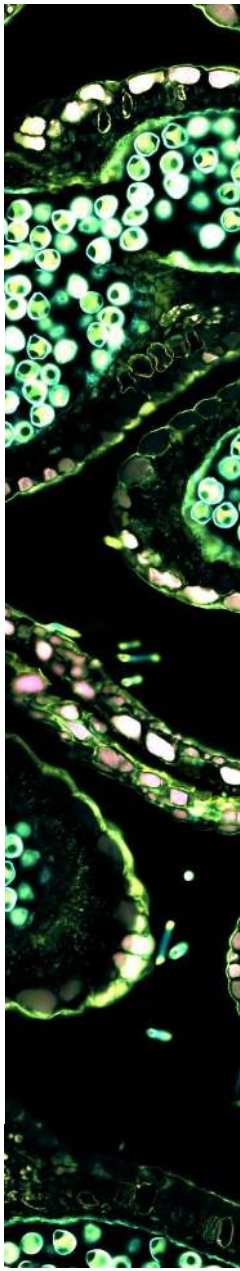
2. Validation of AmphaZ32 results through the use of image analysis

Results

Overall comparison (viable, dead and sterile)







2. Validation of AmphaZ32 results through the use of image analysis

Conclusions

- **~ 14 % higher sterile on average measured** by AmphaZ32

➔ Possible explanations:

1. **Dispersion on microscope slides is higher for sterile than for non-sterile pollen**
2. **A lower sterile % is reflected by a higher maximum viability**

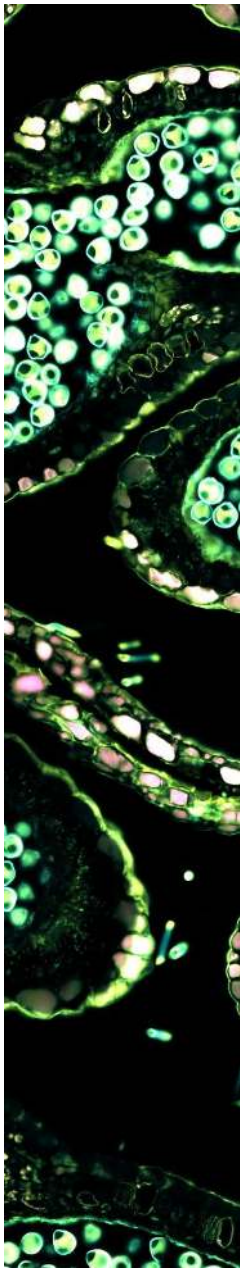
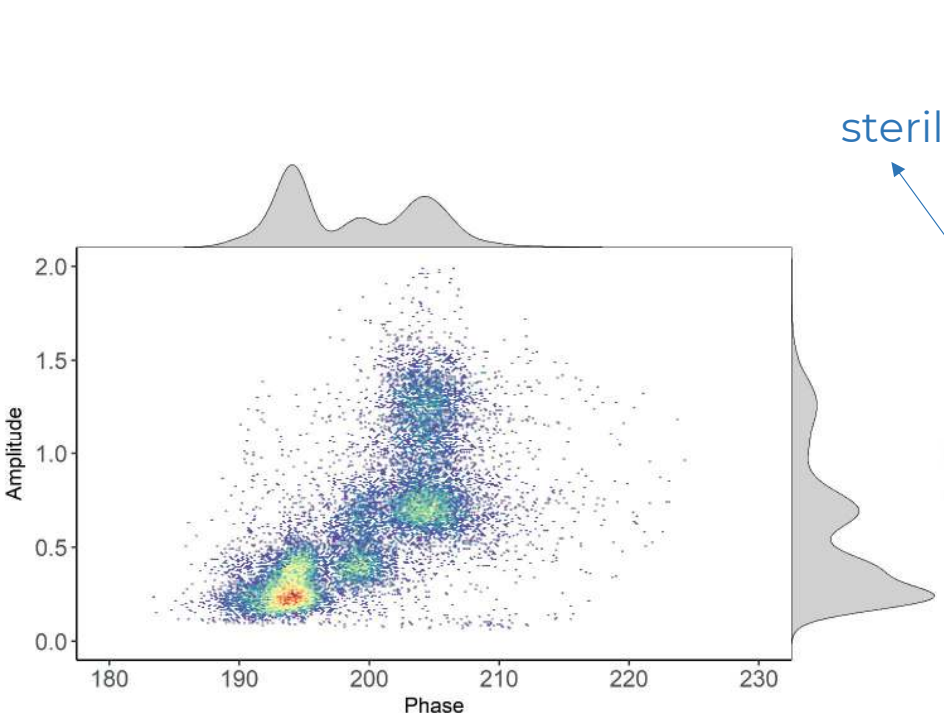
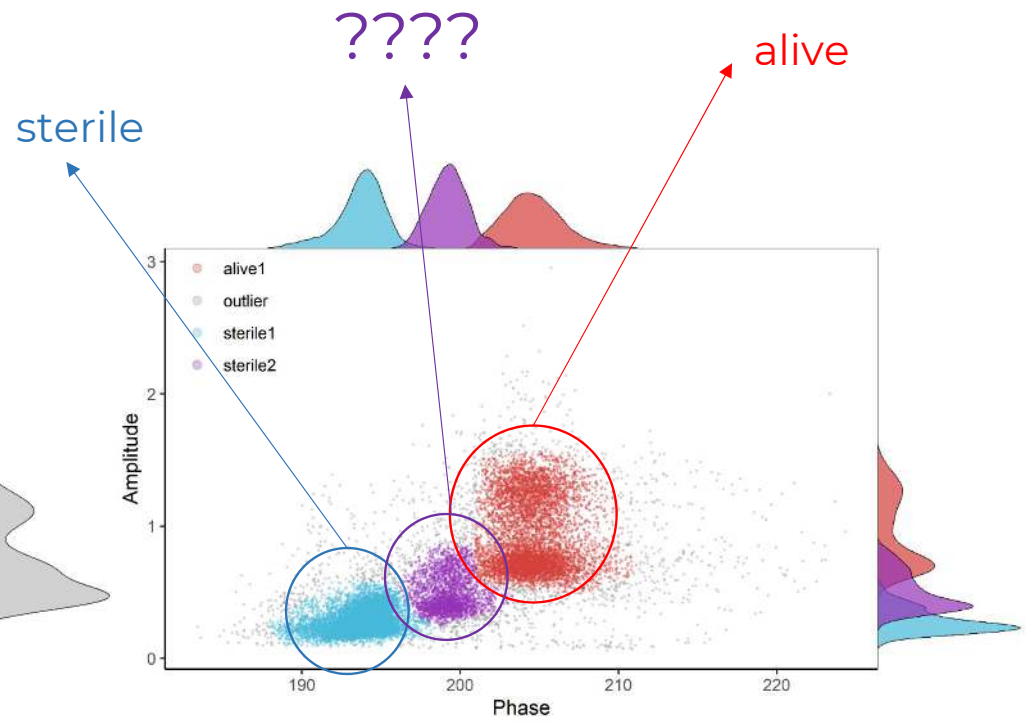


Image analysis and its possible application: data exploration





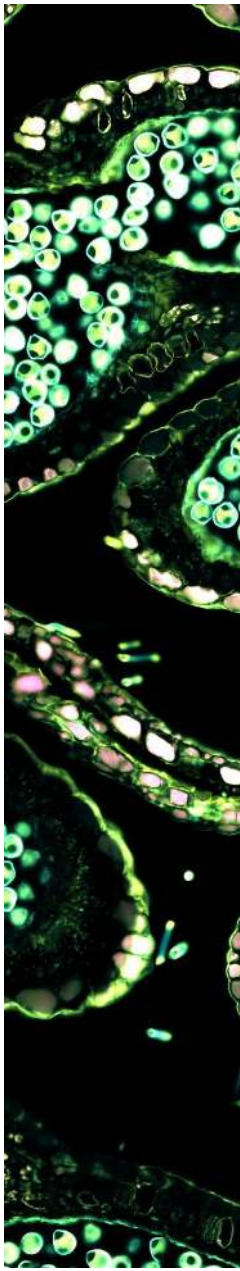
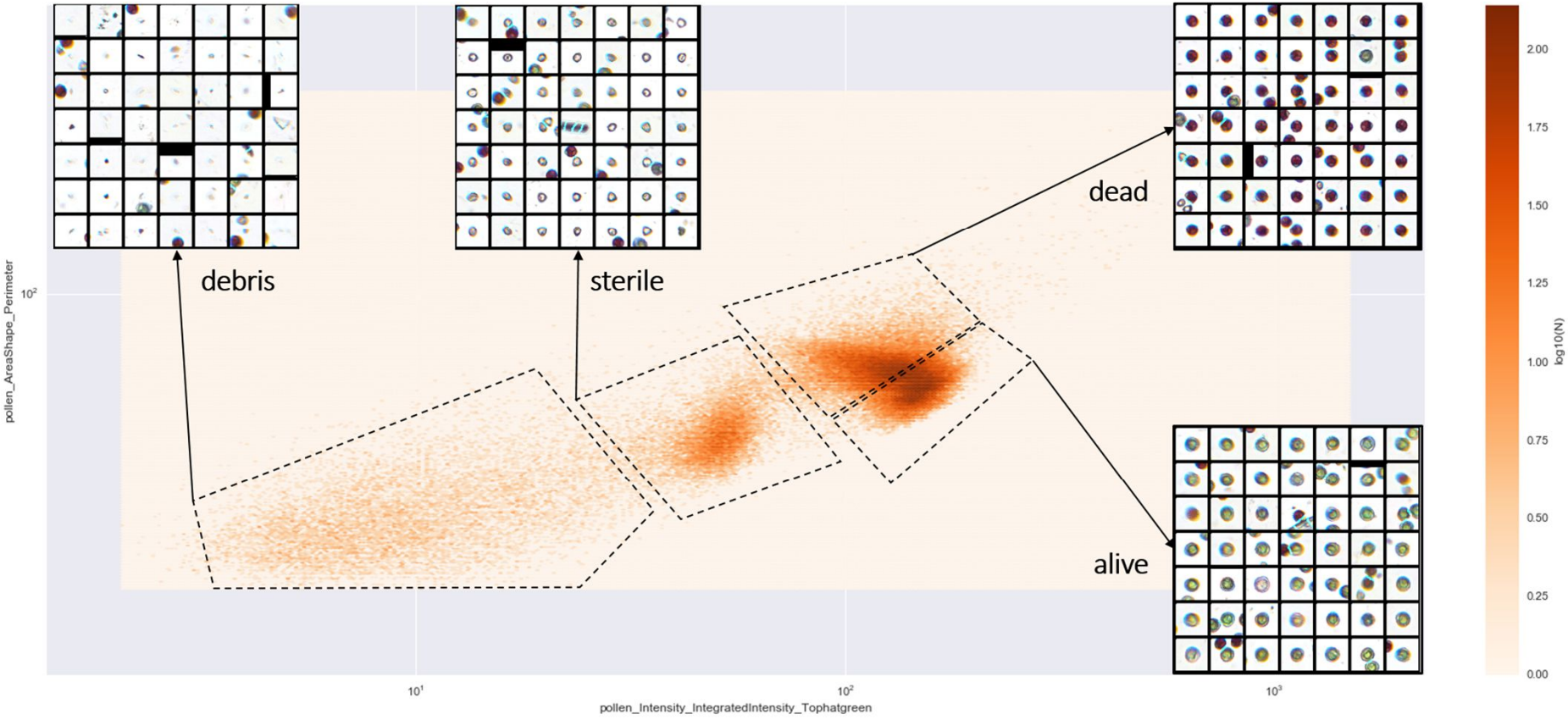
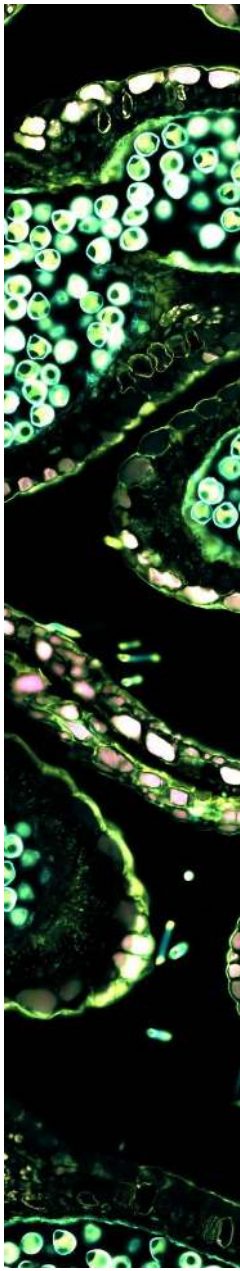


Image analysis and its possible application: data exploration






DBios
Thank for your attention!
myphasys