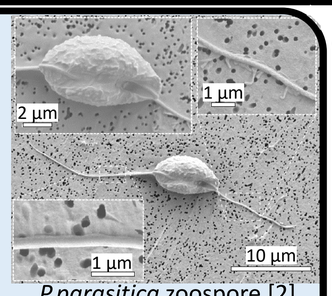
Zoospores swimming in the presence of a root

François-Xavier Gauci
Master Ondes, Atomes et Matière, Université Côte d'Azur

Supervisor: Philippe Thomen
Co-supervisors: Céline Cohen, Xavier Noblin
Institut de Physique de Nice

Context: Oomycetes are microorganisms among which worldwide spread pathogens cause severe damage in crop fields [1]. At a step of their life, they are biflagellate cells called zoospores, swimming through thin films of water to infect roots.

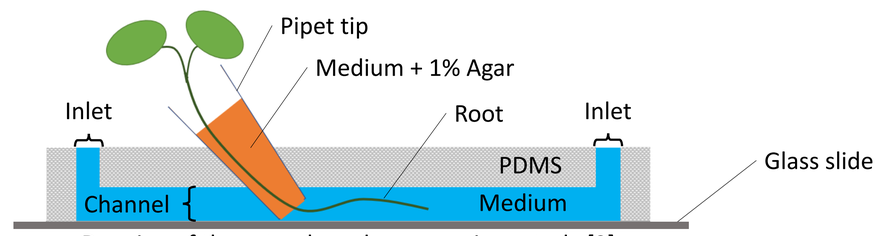


P.parasitica zoospore [2]

Objective: Zoospores-root interaction study consists in identifying signals emitted by a model plant as protons fluxes which likely play a crucial role in *Phytophtora parasitica* zoospores guidance.

Method: By developing a new microfluidic device and performing time-lapse acquisitions, I managed to track zoospores just before infection and characterized their kinematics in the presence of a root.

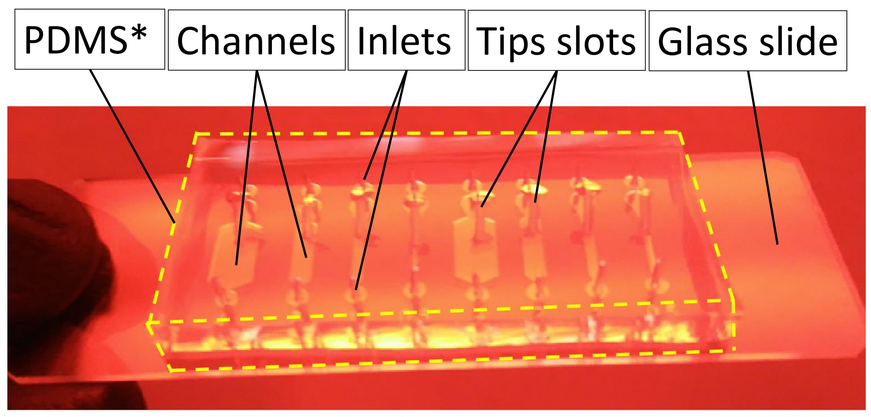
How to capture an infection scene?



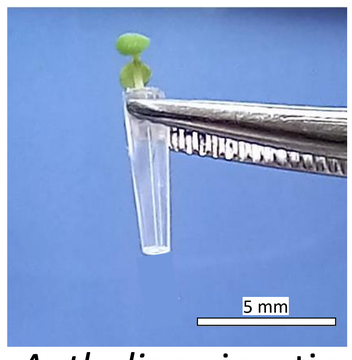
Drawing of the setup based on a previous study [3]

- Making a root grow in a micro channel
- Developing a protocol to enable time-lapse acquisition of zoospores in the presence of a root

Microfluidics: a powerful experimental tool



Microfluidic chip built in the INPHYNI cleanroom



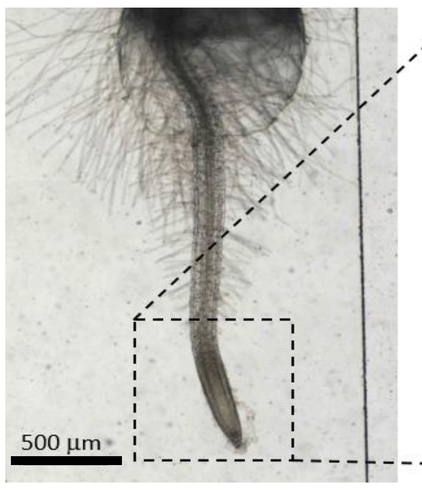
A. thaliana in a tip

- Tips with plants are inserted in the device 3-4 days after seeding
- Roots continue growing in the channels

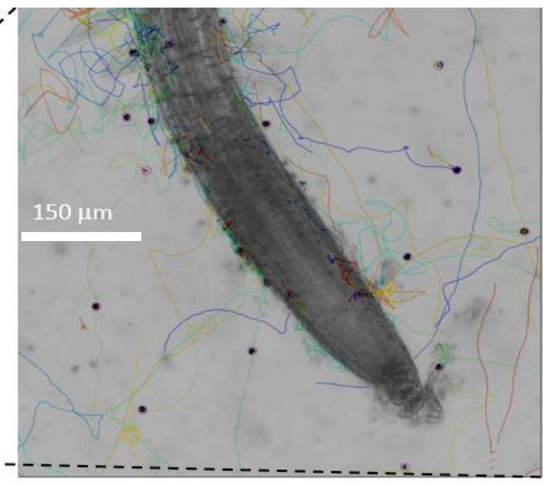
A wide range of benefits:
- A transparent device for microscope observation and acquisition
- A root environment isolated and controlled
- Enables simultaneous acquisitions on different channels

* Polydimethylsiloxane

Tracking zoospores at microscale



Microscope view of a root in the channel (Lens 4x)

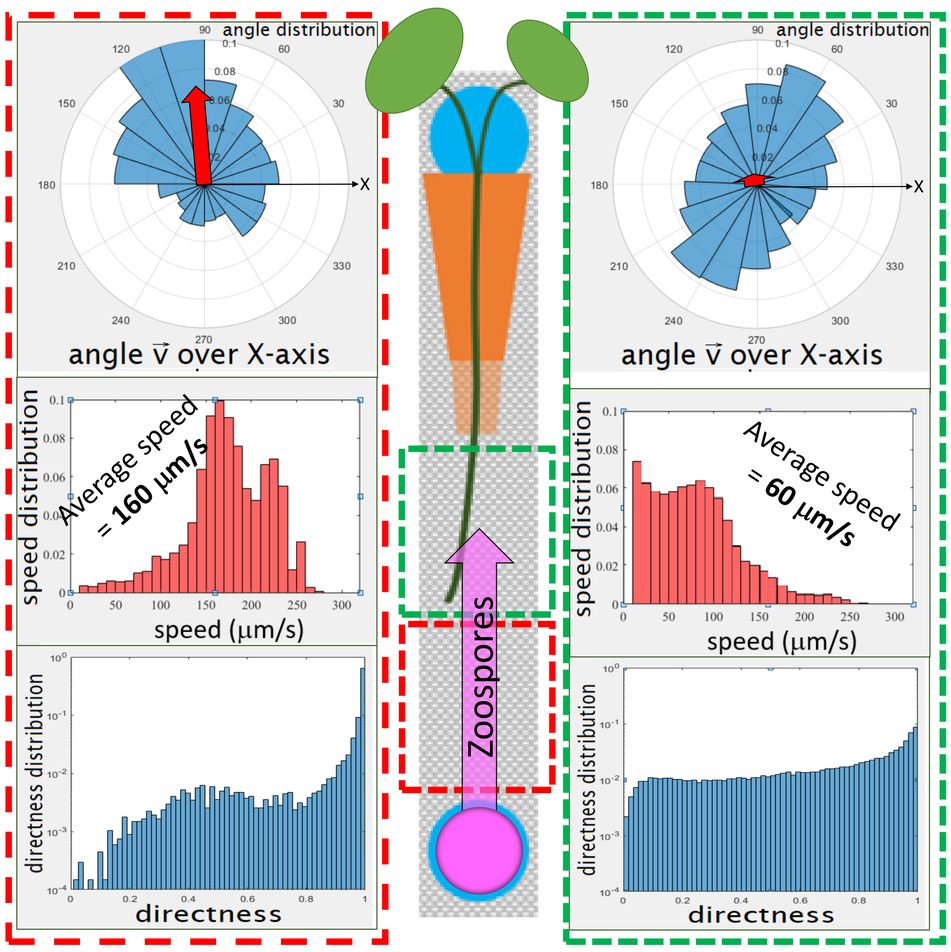


Zoom on the infection zone and superimposed zoospores tracks (Lens 4x)

Acquisition protocol:
- Capturing stacks of pictures
- Removing motionless pixels
- Processing stacks into binary black and white images
- Running TrackMate plugin (ImageJ) with proper input parameters

↳ **to display trajectories and gather positions of zoospores**

A clue that zoospores respond to the root



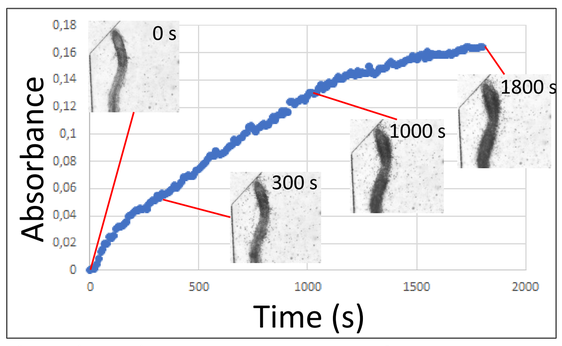
Alteration of zoospores kinematics as they approach a root

- **Direction**
- **Speed**
- **Directness** = $\frac{\text{euclidian length}}{\text{distance travelled}}$

Conclusions & Perspectives

- ✓ Building of a new microfluidic device
- ✓ Development of a replicable protocol
- ✓ Quantitative characterization of zoospores approaching a root:
  - ➢ A speed decrease
  - ➢ A preferential direction disappearing
  - ➢ An increasing number of turning events

Aggregation over time:
- Acquiring every 10 s
- Measuring stack gray value
- Counting zoospores manually on first frames
- Translating intensity in terms of aggregation amount



Plot of the absorbance over time during 30 min

An interdisciplinary project:
- Testing the microfluidic protocol on *A. thaliana* mutants
- Comparing separate channels with different pH

↳ **Verifying the hypothesis: ions fluxes around the roots are signals for zoospore guidance and aggregation before infection**

References

[1] Meng, Y., Q. Zhang, W. Ding, and W. Shan, 2014, MYCOLOGY **5**, 43
[2] Tran, Q., E. Galiana, P. Thomen, C. Cohen, F. Orange, F. Peruani, and X. Noblin, 2021, Coordinated motion of two opposite flagella allows high-speed swimming and active turning in zoospores (submitted)
[3] Massalha, H., E. Korenblum, S. Malitsky, O. Shapiro, and A. Aharoni, 2017, PNAS **117**, 4549–4554