Biophysical isolation of fungus spore in microfluidics

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Project partners: - laboratory Microbiologie Adaptation et Pathogénie, UMR 5240 – Lyon.
- CONIDIA company (https://conidia.fr/champs-etude/maladies-plantes-fongicides/)

Duration : 5 months **Starting date** : march 2022.

This M2 internship will constitute the preliminary work for a PhD subject which is already funded. We are looking for a candidate highly motivated to do a PhD in this field.

Subject :

Botrytis cinerea (*B. cinerea*) is a necrotrophic fungus able to affect more than 1400 plant species, among which several of economic importance such as wine grapes. It is a model fungus in phytopathology, its genome is fully known and numerous molecular tools have been developed to study it. Nevertheless, many aspects of its biology have still to be uncovered. To do so, biologists produce genetically modified strains of *B. cinerea* spores. However, the process to obtained purified sample of the mutated strain (spores with single nucleus) is long (few months scale) and tedious.
The objective of this master internship is to identify the best label-free approach to develop a microfluidic system able to identify and sort spores containing a single nucleus in order to highly speed up the purification process.

Biophysical approaches are well suited for label-free identification (major constraint). For this project, technics based on **cell's size** (such as Deterministic Lateral Displacement (DLD) [1] (fig. a)) and **cell's electrical parameters** (dielectrophoresis (DEP) [2] (fig b) and Electrical Impedance Spectroscopy (EIS) [3]) will be investigated by the intern. The main goal is to determine if there is any correlation between the number of nucleus inside a spore and (i) its size and/or morphology, and (ii) its electrical signature (impedance or Clausius-Mossotti factor).

a) b)

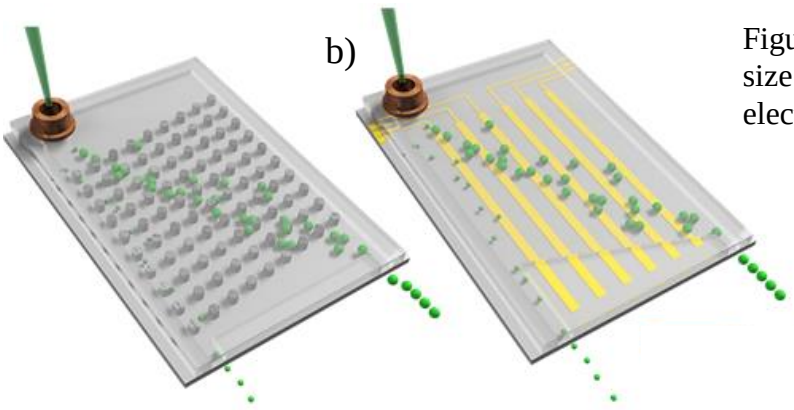

Figure: Sorting based on a) spore size using DLD and b) spore electrical signature using DEP.

Candidate profile:
The student should have a strong taste for experimental work. Knowledge in the area of microfabrication would be a strong asset. Interest on soft matter or biology is of course necessary. In the perspective of the PhD, a candidate with a profile including skills in electronics and/or informatics would be also very valuable as it is plan to design a compact instrument oriented to end-users (biologists).

The M2 candidate will benefit from the technological facilities provided by INL (100m² clean room + 40m² cell culture and bio manipulation) and gain training in micro-nano technologies. It will also be an opportunity for the candidate to strengthen his/her theoretical and practical expertise in the microtechnologies. Finally, the candidate will benefit from the expertise of partners on *B. cinerea*, such as Christophe Bruel from MAP (Microbiologie, Adaptation Et Pathogénie) laboratory.

Any candidacy must be made as soon as possible because the administrative procedure requires 2 months.

Bibliography:
[1] Inglis, D.W., Herman, N., Vesey, G., 2010. Highly accurate deterministic lateral displacement device and its application to purification of fungal spores. Biomicrofluidics 4, 024109.. doi:10.1063/1.3430553

[2] Patel, S., Showers, D., Vedantam, P., Tzeng, T.-R., Qian, S., Xuan, X., 2012. Microfluidic separation of live and dead yeast cells using reservoir-based dielectrophoresis. Biomicrofluidics 6, 034102.. doi:10.1063/1.4732800

[3] Valero, A., Braschler, T., Renaud, P. 2010. A unified approach to dielectric single cell analysis: Impedance and dielectrophoretic force spectroscopy, Lab Chip,10, 2216-2225, doi : 10.1039/C003982A