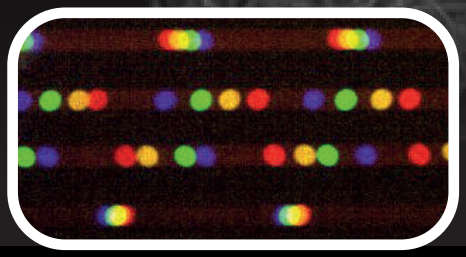
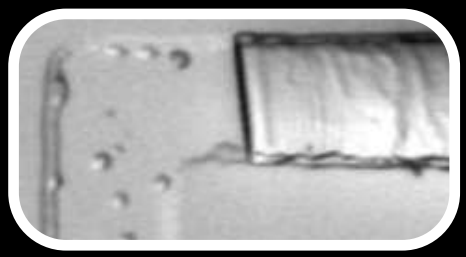
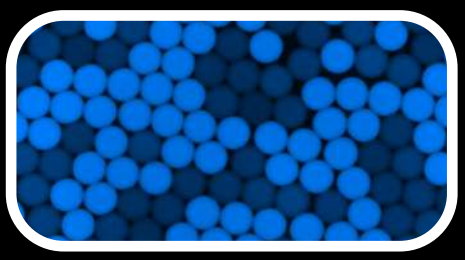
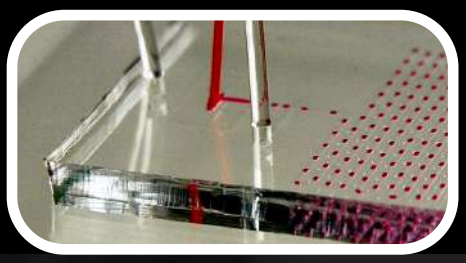
Recent Trends in Microfluidics

workshop @ ENSMAC, Pessac
10 June 2024









Shaping **a local network** for **academics** and **industry** working with **microfluidics**

Biology - Health - Chemistry
Physics - Energy - Materials - ...

Submission of contributions
before 15 April 2024
at https://microflu2024na.sciencesconf.org
talk 10 min + 5 min | **poster** (& flash-com)

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Workshop **Microfluidique en Aquitaine** - 10 Juin 2024 - ENSMAC

08:30		Accueil
08:45		Introduction
09:00	Laurie Iescos	TBA
09:15	Lucas Suire	Electro-mechanical control of Plateau-Rayleigh instability for homogenous multi-cellular production
09:30	Margaux Zollo	Towards microfluidic liquid-liquid extraction using switchable hydrophilicity solvents
09:45	Jean-Christophe Baret	Microfluidics for next gen biotech solution
10:00	Daniel Garcia Ruano	Measuring the volume of single yeast cells using a microfluidic-based fluorescence exclusion method
10:15	Claire-Line Marais	Developing a Drop-based Microfluidic Method for the Analysis of Non-aqueous Plant Cell Fractions
10:30		Pause
11:00	Marc Lagoin	Taylor-Aris dispersion of active particles
11:15	Ludovic Brivady	Optorheology of thin non-Newtonian fluid films
11:30	Jean-Baptiste Salmon	Water potential measurements using water clusters in PDMS microfluidic chips: application to directional drying
11:45	Zhansaya Aitkhozha	Microfluidics-based experimental study of the use of yield stress fluids to improve organic pollutant removal from contaminated soils
12:00	Abdi Mirgissa Kaba	Novel Confocal Absorbance-measurement Platform for Ultrahigh-throughput Screening of Enzyme Variants in Droplet-based Microfluidics
12:15	Léon Rembotte	Image-activated Organoid Cytometry and Acoustic Sorting
12:30		Repas + Posters
14:00	Adèle Varnier	TBA
14:15	Alisa Svirina	Broadband spectroscopy for visualising electrochemical reactions in transparent microfluidic devices
14:30	Samuel Marre	La microfluidique haute pression : De la thermodynamique à la biogéochimie, un outil pour l'étude des milieux fluides réactifs en pression et en température
14:45	Antoine Aubret	Control of colloidal crystallization under confinement with light
15:00	Manon Chargy	Développement d'une puce microfluidique de PCR quantitative pour suivre la dynamique d'une communauté synthétique de microorganismes ayant un potentiel effet de biocontrôle contre le mildiou de la vigne
15:15	Mélanie Gillard	High-throughput antimicrobial peptide screening using droplet microfluidics
15:30		pause
16:00	Julien Renaudeau	Osmotically-driven flows and passive loading in artificial phloem: a microfluidic approach
16:15	treefrog	TBA
16:30	Jacques Leng	3D Anderson localization of acoustic waves
16:45	Stéphane Chevalier	Spectroelectrochemical Imaging in Microfluidic Electrochemical Devices: a pathway to reach the micromolar scale
17:00	Olga Fuentes	Life Cycle Assessment of Magnetite Production Using Microfluidic Devices: Movingfrom the Laboratory to Industrial Scale
17:15	Fatma Ercicek	In situ microfluidic investigations of APIs crystallization dynamics in scCO2: From thermodynamic equilibrium to growth kinetics
17:30		Table ronde
18:00		End

Abstracts

Oral presentations

Recent Trends in Microfluidics
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Electro-mechanical control of Plateau-Rayleigh instability for homogenous multi-cellular production

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In the BioImaging & Optofluidics team we have developed a micro-fluidic encapsulation technique enabling high-throughput production of multi-cellular systems. These assemblies, such as organoids that recapitulate certain organ functions, are of great interest and have numerous applications in fields such as tissue engineering, oncology and regenerative medicine [1]. The encapsulation technique is based on Rayleigh-Plateau instability that explains how a falling stream of fluid breaks up into smaller droplets with the same volume but reduced surface area [2]. In our configuration, our system typically produces droplets of around 300-500 µm in diameter at a rate of 5000 capsules per second. Yet, without external control of the instability, coalescence occurs and the resulting diameter distribution is relatively broad.

As we are seeking to achieve homogenous aggregate production for biological applications, it is pivotal to control the fragmentation of the jet with an external excitation.

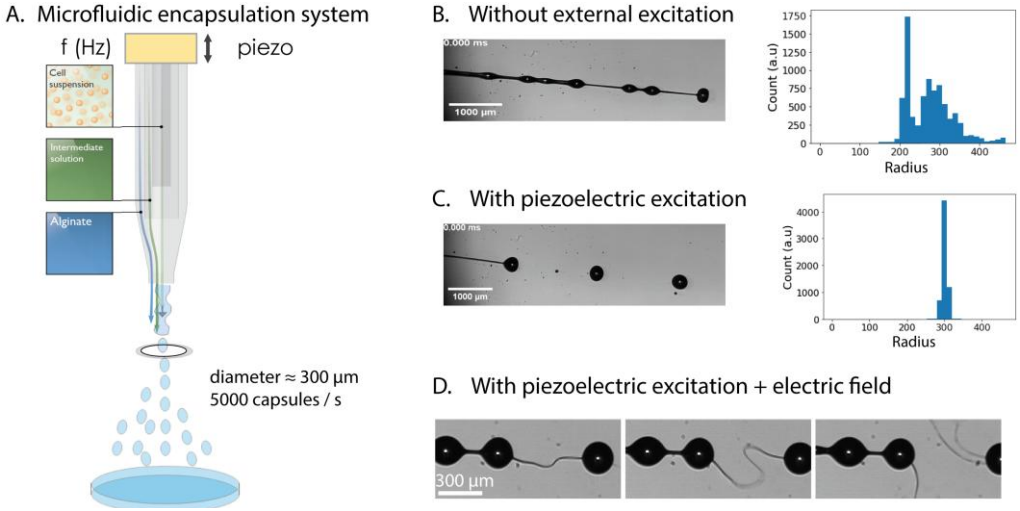

Figure 1: (A) Sketch of the microfluidic encapsulation system. (B)Image of the instability without external excitation and the resulting droplet diameter distribution. (C) Same as (B) but with a piezoelectric excitation at 2500 Hz. (D) Application of an additional electric field at 2500 V to reduce the coalescence by breaking the viscous filament.

In the past, several strategies had been developed to perform an external control of the Plateau-Rayleigh instability including electric field, acoustic waves or piezoelectric actuation [3]. Here, we show that a combination of high-electric field and piezoelectric actuation lead to an homogeneous distribution of sizes over a broad range of diameters.

First, we show that piezoelectric excitation provides an efficient method to obtain an homogeneous distribution of droplet diameters (see figure 1.B with f=2500 Hz). By changing the excitation frequency (between 2 and 6 kHz), droplet diameter can be adjusted over a moderate range (500-600 µm). Outside this range, mode selection with the piezoelectric is not effective and coalescence prevails. Secondly, by adding a strong continuous electric field, we observe the mode selection range of the piezo can be extended (2 to 9 kHz) providing a controlled distribution of droplet diameters between 400 and 600 µm.

This combination of these two external excitation (piezoelectric and electric) offers optimum control of Rayleigh-Plateau instability, enabling us to produce highly homogeneous and adjustable capsules.

[1] Alessandri, et al. (2013). *Proceedings of the National Academy of Sciences*, *110*(37), 14843-14848.
[2] Eggers, J. (1997). *Reviews of modern physics*, *69*(3), 865.
[3] Doméjean, H. (2014). (Doctoral dissertation, Paris 6).

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Towards microfluidic separation processes using Switchable Hydrophilicity Solvents

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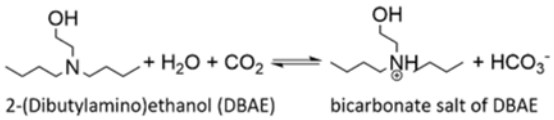
Solvent engineering aims at controlling the solubility and transport properties of a solvent medium, to optimize the chemical or physical processes performed. Among the strategies of Solvent Engineering, reversible CO_2-switchable hydrophilicity solvents (CO_2-SHS) represent a promising route to reversibly switch the properties of a solvent.

The final goal of this work is to combine microfluidic technologies and CO_2-SHSs to perform liquid-liquid separation processes at microscale and in optimized experimental conditions. 2,2-Dibutylaminoethanol (DBAE) was chosen as this solvent is able to switch from a tertiary amine (hydrophobic form), to the corresponding bicarbonate salt (hydrophilic form) at room conditions[1]:



We first developed microfluidic chips made of poly(dimethylsiloxane) (PDMS) using soft lithography (typical channel height 10-50µm), PDMS being the material of choice, not only for the versatility and simplicity of microfabrication, but also for its permeability to gasses. We observed that DBAE is able to extract uncross-linked PDMS oligomers from the PDMS matrix of the chip as other organic solvents[2]; this phenomenon interfered with our observations. To overcome this drawback, we developed protocols to wash-out these uncross-linked oligomers leading to PDMS chips fully compatible with DBAE. We then developed two-level PDMS chips. This allowed us to impose either a CO_2 or an N_2 flow in a channel superimposed to a fluidic channel in which the DBAE and water were present. Because of the permeability of PDMS to gasses, we were able to induce the phase change of the SHS and modulate its solubility into water. We tested two different conditions: (a) a static condition with a stable interface between DBAE and water and a dynamic condition with a train of water droplets in a continuous DBAE stream (see Fig. 1a and 1b respectively). We are currently exploiting these microfluidic experiments to design a liquid-liquid separation process for the extraction of soybean oil in a microchannel.

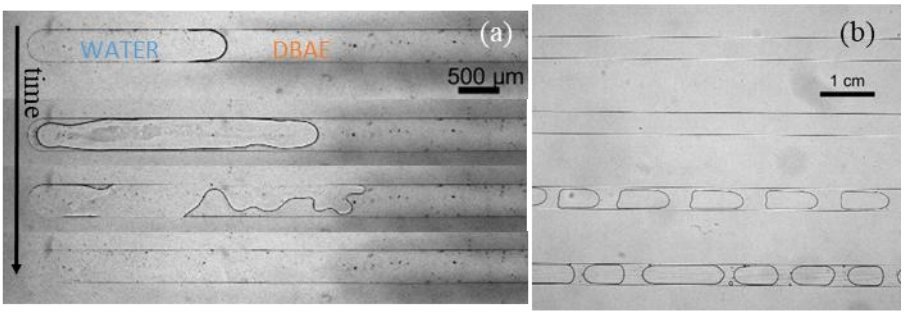


Figure 1 : DBAE hydrophilicity switch in a microfluidic static (a) and dynamic (b) condition.

(1) Jessop P., *Aldrichimica Acta*, **2015**, 48, 1.
(2) Lee J. N. et al., *Analytical Chemistry*, **2003**, 75, 23.

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Microfluidics for next gen biotech solution

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Over the past twenty years, microfluidics has offered new means to miniaturize and automatize biochemical and cell-based assays. The technology is now key to address pressing problems, making use of its potential to screen large libraries of compounds and organisms. We apply this technology to the biodegradation of plastics, the screening of planktons for biomass production and lately for the assembly of functional synthetic cell.

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Measuring the volume of single yeast cells using a microfluidic-based fluorescence exclusion method

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The regulation of cell volume and its role in cell biology have been the focus of in-depth investigation, providing important insights into this central feature of living cells. However, the majority of studies on cell size to date rely on various proxies for cell volume or on highly specialized tools that are not easily accessible to most research teams. To overcome these limitations, a method combining Fluorescence eXclusion (FXm) with microfluidic technologies was recently implemented for the direct and reliable determination of mammalian cell volume (1). However, whether FXm was compatible with much smaller cells such as yeast, one of the most common genetic models for deciphering the mechanisms of cell size control, remained unknown. We therefore adapted the FXm microfluidic devices to small cells and demonstrated that this approach makes it possible to accurately and reproducibly measure the volumes of individual fission yeast cells. Furthermore, our results highlight the power of FXm, which challenges the conclusions that are obtained with more traditional methods, in particular when comparing the sizes of yeast cells that have different shapes and aspect ratios. Finally, we showed that FXm is compatible with the real-time monitoring of other intracellular fluorescent markers and allows for tracking the volume of single cells with high temporal resolution, notably upon changes in their environment (2, 3). Altogether, this work demonstrates how coupling yeast genetics with FXm will bring a new understanding of cell growth and cell volume regulation in various biological contexts.

1) Cadart, C., Zlotek-Zlotkiewicz, E., Venkova, L., Thouvenin, O., Racine, V., Le Berre, M., Monnier, S. and Piel, M. *Methods Cell Biol.* 139, 103–120 (2017).
2) García-Ruano, D., Venkova, L., Jain, A., Ryan, J.C., Balasubramaniam, V.R., Piel, M. and Coudreuse, D. *J Cell Sci* 135:jcs259392 (2022)
3) Venkova, L., Garcia-Ruano, D., Jain, A., Charvin, G. and Coudreuse D. *Methods Mol Biol. in press*

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Developing a Drop-based Microfluidic Method for the Analysis of Non-aqueous Plant Cell Fractions

Claire-Line Marais[1, 2], Bertrand Beauvoit[1], Martine Dieuaide-Noubhani[1], Yves Gibon[1, 2], Mickaël Maucourt[1, 2], Pierre Petriacq[1, 2]

[1]Univ. Bordeaux, INRAE, UMR1332 BFP, 33882 Villenave d'Ornon, France ;
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Seduced by the possibilities offered by microfluidics, the Meta team of the UMR 1332 BFP (INRAE – University of Bordeaux) has been developing a microfluidics platform since 2019 within the Bordeaux Metabolome facility. Soon to be operational, this platform will enable us to carry out all microfluidics steps, from creating chips by laser photolithography or high-resolution 3D printing methods to performing experiments.
The two main areas of research are the study of plant enzymes and the metabolome of organelles. For the second axis, the choice was made to study mitochondria. Plant mitochondria not only provide energy but also building blocks to biomass construction and defence. Despite its importance, the plant mitochondria metabolome remains largely unknown. Non-aqueous fractionation (NAF) is already used to analyse the metabolome of plastid, vacuole, and cytosol of plant cells. However, this method is unable to provide an enriched mitochondrial fraction due to the small size of these organelles and their low density when dehydrated. To overcome this limitation, we are developing a drop-based microfluidic method allowing us to sort mitochondria with high purity and yield. The enriched fraction will then be analysed using mass spectrometry-based targeted and untargeted metabolomic approaches.
The versatility of our manufacturing tools allows us to imagine and easily create chips and equipment to develop new approaches and overcome scientific obstacles.

Taylor-Aris dispersion of active particles

Guirec de Tournemire, Marc Lagoin, Ahmad Badr, Juliette Lacherez, Nicolas Fares, Antoine Allard, Yacine Amarouchene, Thomas Salez
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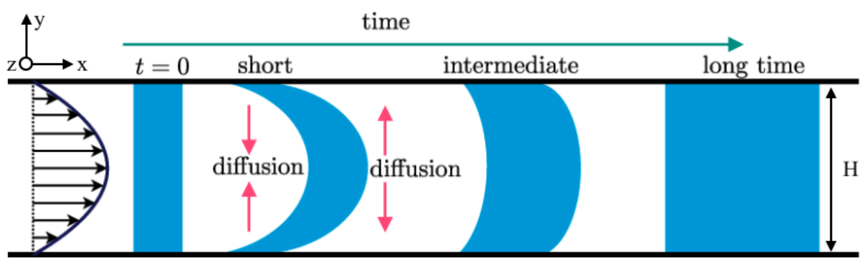
Abstract

In a shear flow, the diffusion of particles gets enhanced by the coupling between advection and thermal diffusion. This is the so-called and classical Taylor-Aris dispersion. Over the past decades, in the context of active matter, living microswimmers have been abundantly studied, both experimentally and theoretically. Interestingly, their motion at large time scales has been shown to be diffusive-like with an effective diffusion coefficient controlled by a coupling between the raw diffusive and active properties. However, such swimmers often experience external flows in natural settings. Therefore, combining Taylor-Aris dispersion and activity appears as an important but open problem. Here, we thus study alive chlamydomonas within microfluidic flows and quantitatively investigate their effective dispersion.

Taylor-Aris dispersion of passive particles

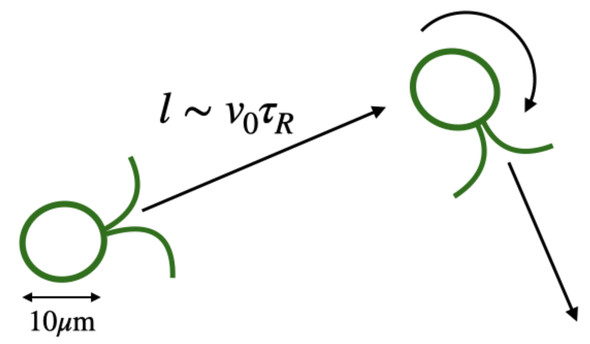


$$D_{\mathrm{TA}} = D_0(1 + \alpha \mathrm{Pe}^2)$$

$$\begin{cases} \mathrm{Pe} \equiv \frac{UH}{D_0} = \frac{H^3}{4D_0\eta L}\Delta P \\ D_0 = \frac{k_B T}{6\pi\eta R} \end{cases}$$

H= 180μm
U : mean velocity of the flow
η : fluid viscosity
R : radius of the particle

Active particles



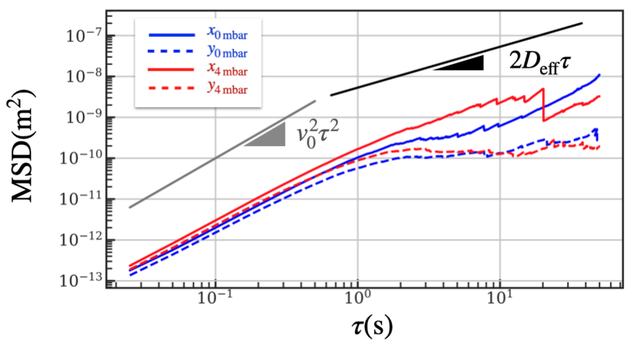
$$D_{\mathrm{eff}} = D_0 + D_{\mathrm{swim}}$$

with $\mathrm{D_{swim}} \sim \frac{l^2}{2\tau_R}$
τ_{R} : reorientation time
v_0 : average velocity of swimming

Question of Interest

How does shear affects effective diffusion of active particles ? $D_{\mathrm{TA}} = D_{\mathrm{eff}}(1 + \alpha \mathrm{Pe}^2)$?

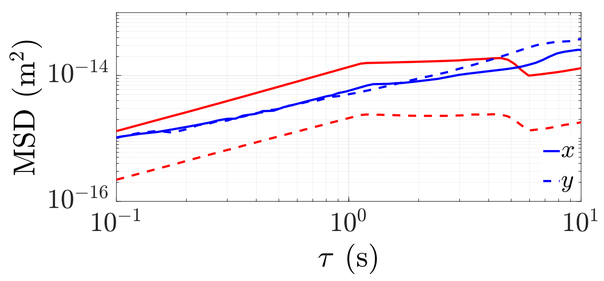
Averages

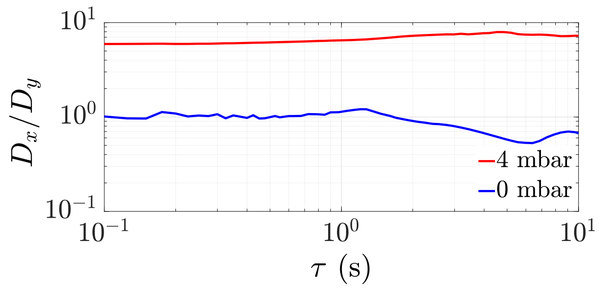


D_y unaffected by the flow

D_x enhanced by the flow

Individual dispersion





Diffusion may depend also on the particle choice

D_x/D_y increases with the pressure

Perspective : characterization of α to achieve the full understanding of the Active Taylor Dispersion :

$$\frac{D_x}{D_y} - 1 = \alpha \mathrm{Pe}^2$$

References

[1] Peng, Z., Brady, J. F. (2020). Upstream swimming and Taylor dispersion of active Brownian particles. Physical Review Fluids, 5(7), 073102.
[2] Sevilla, F. J., Gómez Nava, L. A. (2014). Theory of diffusion of active particles that move at constant speed in two dimensions. Physical Review E, 90(2), 022130.

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Optorheology of thin non-Newtonian fluid films

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Complex fluids in confinement and at interfaces, and their puzzling rheology at small scales, is a modern challenge of soft-condensed-matter physics, with direct implications in nanotechnology and industry. How do these systems flow or deform at scales comparable to the ones of their inner components still leaves a lot of open fundamental and applied questions in out-of-equilibrium conditions. Examples include the stability of nanomechanical data-storage devices, the transient rheology during solvent evaporation of ink (related to the so-called “coffee-ring effect”) and paint deposition (e.g. crust/crack formation) when the film thickness is well below the capillary length. Even if many types of reliable rheometers exist, most of them cannot be used on thin samples or non-equilibrium systems, often because mechanical contact may induce pollution and modify the local structure. To overcome these difficulties, we implement here a local, active, fast and contactless optical approach [1], which consists in deforming complex fluid interfaces through optical radiation pressure from a pump laser, and further analyzing the dynamics of this deformation using the Newton rings pattern (Fig. 1a). We combine this method to modified lubrication models [2] in order to investigate the non-Newtonian rheology of thin films composed of small-molecular-weight polyacrylamide (PAAm, 5–6 M) diluted in DI water, at different concentrations. Single-shot experiments offer the opportunity to characterize the size-dependent viscoelasticity (Fig. 1b), while temporally-modulated laser excitations provide a way to measure precisely the stress-dependent properties such as shear thinning.

[1] G. Verma, H. Chesneau, H. Chraïbi, U. Delabre, R. Wunenburger, & J. P. Delville. Contactless thin-film rheology unveiled by laser-induced nanoscale interface dynamics. Soft Matter, **16**, 7904 (2020).
[2] M. Ilton, M. M. P. Couchman, C. Gerbelot, M. Benzaquen, P. D. Fowler, H. A. Stone, E. Raphaël, K. Dalnoki-Veress, and T. Salez. Capillary levelling of freestanding liquid nanofims. Physical Review Letters, **117** 167801 (2016).

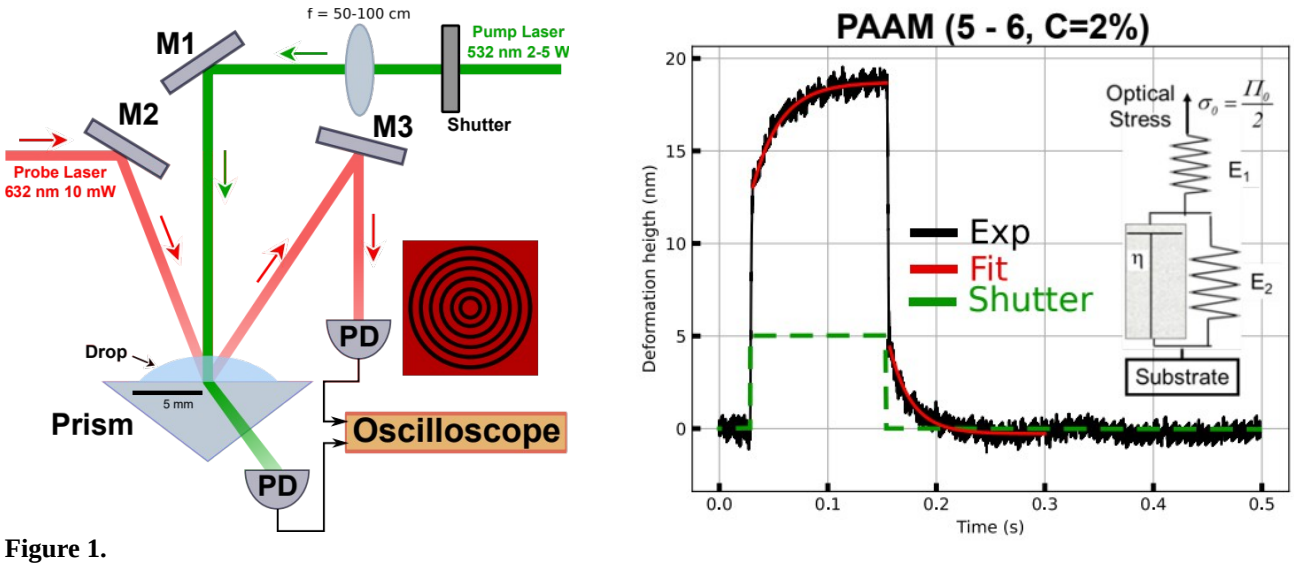

Figure 1.
(a) Optorheology setup: the radiation pressure of a continuous green pump laser beam is used to locally deform the free surface of a thin sessile droplet deposited on a prism. The relaxation dynamics is then probed by interferometry with a loosely focused continuous red He–Ne laser. (b) Example of deformation height versus time, for a PAAM polymeric solution exhibiting a viscoelastic response.

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Water potential measurements using water clusters in PDMS microfluidic chips: application to directional drying

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We have developed a PDMS microfluidic chip to study the directional drying of a colloidal dispersion confined in a channel. Our measurements on a dispersion of silica nanoparticles (diameter 22 nm) once again revealed the phenomenology commonly observed for such systems: the formation of a porous solid with linear growth in the channel at short times, slowing down at longer times as the evaporation rate decreases.

In addition to these observations, we report original measurements using hydrophilic filler naturally present in the PDMS formulation used (Sylgard-184). When the PDMS matrix is in contact with water, water molecules pool around these hydrophilic sites, resulting in the formation of microscopic water clusters whose size depends on the water potential. We have used these water clusters to estimate the water potential profile in the channel as the porous solid grows. Using a transport model, we then linked these water potential measurements to the hydraulic permeability of the porous colloidal solid.

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Microfluidics-based experimental study of the use of yield stress fluids to improve organic pollutant removal from contaminated soils

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[b] *I2M, Arts et Métiers Institute of Technology, ENSAM, Esplanade des Arts et Métiers, 33405 Talence Cedex, France*
[c] *I2M, CNRS, Esplanade des Arts et Métiers, 33405 Talence Cedex, France*

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The remediation of polluted soils by the injection of complex fluids is a cost-effective strategy allowing in situ decontamination with minimal environmental and economic impact. The use of polymers such as Xanthan Gum and polyacrylamide in hydrolyzed form (HPAM) allows to increase the efficiency of the macroscopic scanning of a pollutant thanks to the optimization of the viscosity ratio of the injected and displaced fluids[1]. Although significant progress in this field has recently been made, the dynamics of immiscible flows of pollutants by a shear-thinning fluid remains very limited. This can be explained by the difficulties of assessing the influence of the injection pressure of the polymer, the dynamic viscosities and the wettability at the pore scale.
In this work, a series of microfluidic experiments (FIG. 1) were performed to identify those microscopic mechanisms of displacement and to quantify their impact on scanning efficiency. In order to achieve this goal, concentrated xanthan gum solutions with concentrations ranging from 3,000 to 7,000 ppm developing a yield stress will be used as blocking agents. The yield stress fluid is injected through previously "Lab on Rock" microfluidic chips at their residual pollutant saturation. We will present an overview on recent experimental data and the theoretical analysis on this topic.

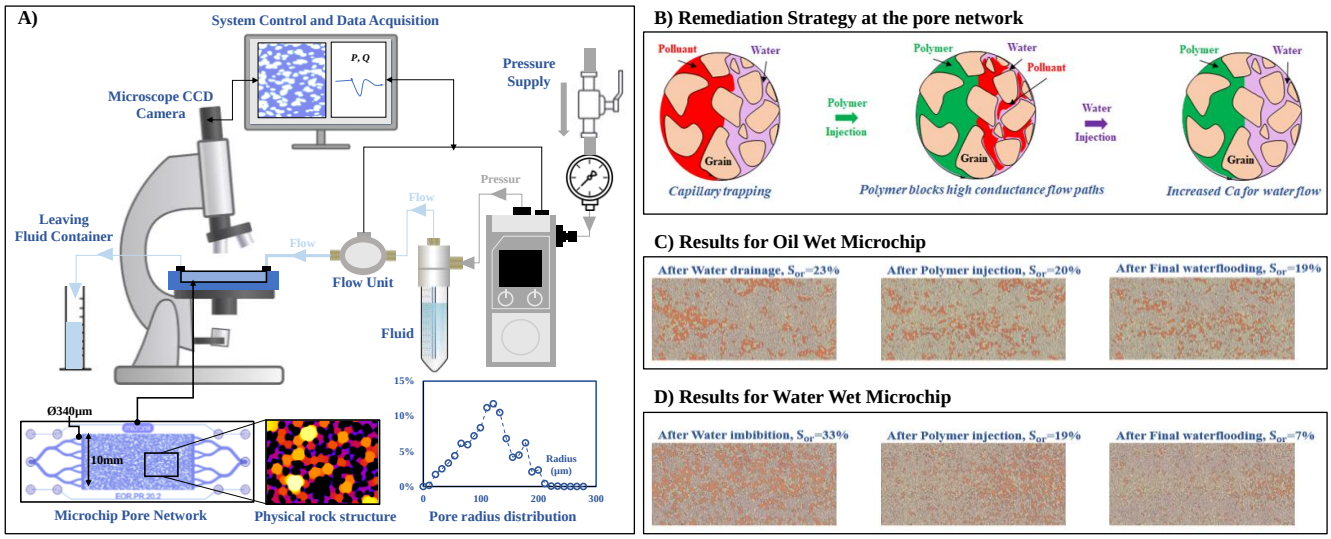

FIG. 1. A) Schematic drawing of the experimental apparatus and the microfluidic borosilicate glass chips (based on the drawing on the website of Micronit Company). The chips are 10 mm × 20 mm: Physical rock structure with a 150 µm wide crack, B) Remediation strategy and injection steps, C) Oil wet microchip pore network: Results of residual oil saturation S_{or} after Water Drainage, after Polymer Injection and after Final Waterflooding, D) Water wet microchip pore network: Results of residual oil saturation S_{or} after Water Imbibition, after Polymer Injection and after Final Waterflooding.

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Recent Trends in Microfluidics
workshop @ ENSMAC, Talence, 10 June 2024

Novel Confocal Absorbance-measurement Platform for Ultrahigh-throughput Screening of Enzyme Variants in Droplet-based Microfluidics

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Directed evolution (DE), a process of enzyme-activity improvement via iterative variant generation, screening, and selection, has benefited greatly from advances in droplet microfluidics, which allow for rapid screening of large enzyme libraries.[1] In such a process, water-in-oil emulsions with a $>10^7$-fold smaller volume than a conventional 96-well plate, compartmentalize an enzyme-expressing gene with a fluorogenic substrate for a high-throughput (~2 kHz) fluorescence-activated droplet sorting based on the enzyme performance. However, these assays are not available for many enzymatic systems.[2]

To overcome this limitation, alternative detection and sorting methods based on absorbance have been recently reported.[2] Several chromogenic substrates can be tested using these techniques, unlocking previously unaccessed sequences. Nevertheless, these methods suffer from low sorting frequency (< 1 kHz) due to the large size of droplets (> 75 pL) and poor sensitivity as a result of scattering from droplet edges. To overcome these limitations, we introduce a custom confocal absorbance-activated droplet sorting (AADS) platform that confines the incident light to a smaller detection volume, substantially minimizing scattering and enabling enhanced detection from sub-75-pL droplets. Furthermore, our setup allows a simpler chip design and measurement from any area along the channel, a stark contrast to previous works where an optical fiber inserted perpendicular to the channel complicates chip preparation and defines detection area.

The setup showed excellent sensitivity and linearity (R^2=0.98), examined by measuring readouts from droplets containing a pure Bilirubin Oxidase (BOD) enzyme compartmentalized with concentrations of 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) ranging from 0 to 4 mM. Moreover, absorbance was accurately measured from droplets as small as 25 pL, 0.33× the smallest previously reported droplet.[2] Furthermore, dielectrophoretic sorting of 50-pL droplet population at ultrahigh frequencies (e.g., 2 kHz) showed remarkable efficiency of 99% that exceeds all previous AADS reports and par the widely utilized fluorescence-activated droplet sorting.

Ultimately, we aim to use our platform for ultrahigh throughput redox-potential estimation of new enzymes. A low redox potential bacterial BOD (+0.34V vs. Ag/AgCl at pH 7)[3] will serve as a model to identify a BOD with higher potential (~+0.5V) for efficient use in biofuel cells. We anticipate that our screening platform will be an important tool for the advancement of DE in droplet-based microfluidics.

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Recent Trends in Microfluidics
workshop @ ENSMAC, Talence, 10 June 2024

Image-activated Organoid Cytometry and Acoustic Sorting

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Three-dimensional cell models, such as organoids and spheroids, have become indispensable tools for studying tissue-level biology.[1] Their ability to recapitulate physiological interactions between cells and their mechanical environment has recently led to pioneering discoveries in cancer and developmental biology. However, manual analysis methods are time-consuming, limiting their widespread use in biology laboratories for statistically-relevant studies. Here, we introduce an automated approach for high-throughput characterization and sorting of submillimetric multicellular aggregates. Our system utilizes real-time image processing to assess morphological and phenotypical traits in up to 10 aggregates per second. These aggregates are then individually encapsulated into water drops, whose trajectories are controlled using acoustic forces. We demonstrate our system's performance by achieving precise selection of homogeneous-sized spheroid samples and high-purity separation of aggregates composed of different cell types. We anticipate that this work lays the foundation for standardized drug screening on organoids, promising accelerated progress in biomedical research.

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Broadband spectroscopy for visualising electrochemical reactions in transparent microfluidic devices

Microfluidic devices have recently attracted the attention of many researchers as they offer many advantages as devices for various types of analysis and are also used as electrochemical devices, for example redox flow batteries (RFB). Some of the prospects for using such devices include reduction of the price of the analysis and determination of very low concentrations thanks to the reduced sample volume.

In this study, we present 2 approaches to visualise electrochemical reactions in microfluidic devices using IR and UV-vis spectroscopy techniques. Firstly, by introducing a Y-shaped microfluidic channel design with platinum electrodes positioned along the sidewalls, we aim to facilitate real-time imaging of reactions, and address the challenge of electrodes blocking the view, which is common in traditional setups where electrodes are placed at the bottom. Being able to see through the channel will ease data processing since we will have more pixels to extract the data from. Another way that we suggest to overcome this problem is to incorporate transparent conductive materials such as indium tin oxide (ITO) in combination with ultra-thin (under 50 nm) Pt layers. The two proposed geometries will help to quantify diffusion coefficient, kinetics and thermal effects of the reaction. (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl also called TEMPO and Methyl viologen dichloride were chosen as chemicals for this study and their electrochemical and chemical properties were assessed.

Recent Trends in Microfluidics
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La microfluidique haute pression : De la thermodynamique à la biogéochimie, un outil pour l'étude des milieux fluides réactifs en pression et en température

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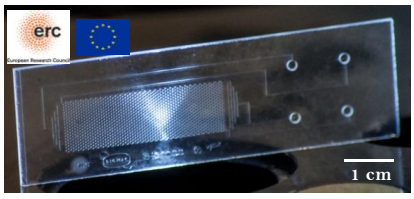
Au cours des quinze dernières années, un nouveau champ d'investigation s'est développé autour de la microfluidique haute pression. Il est basé sur l'idée de combiner les avantages de la microfluidique (réduction en taille, criblage rapide, analyses in situ, reproductibilité, contrôle de l'hydrodynamique, amélioration des transferts thermiques et de matière, faible consommation de réactifs pendant les phases d'optimisation, etc.) avec les propriétés des systèmes fluides employés dans des conditions de haute pression et haute température (mise en œuvre de procédés hydro- et solvothermaux, études des écoulements de géofluides en milieux poreux modèles, biologie en conditions extrêmes, fluides supercritiques, etc.). Ces nouveaux outils permettent d'étudier plus finement les phénomènes se déroulant aux petites échelles et se positionnent de manière complémentaire des approches classiques utilisant soit des réacteurs batch macroscopiques soit des cellules à enclume de diamant. Le développement de cette technologie microfluidique haute pression a permis d'ouvrir de nombreuses opportunités pour étudier et caractériser un grand nombre de processus / procédés utilisant les fluides sous haute pression (jusqu'à quelques centaines de bar à l'heure actuelle). Ces outils permettent de s'affranchir des limitations des montages expérimentaux classiques qui sont généralement « aveugles » car ne permettant pas d'intégrer facilement des techniques de caractérisation *in situ* pour étudier en temps réel les phénomènes couplés (thermodynamique, hydrodynamique, transport réactif, chimique et microbiologique) se déroulant aux petites échelles.

Dans cette présentation, nous allons tout d'abord détailler les technologies disponibles pour la fabrication des microréacteurs haute pression, puis nous aborderons leur utilisation dans plusieurs applications comme : (i) l'étude des propriétés thermodynamiques de mélanges fluidiques complexes, (ii) les micro-mélanges en conditions turbulentes, (iii) la cristallisation de molécules organiques assistés au CO_2 supercritique et (iv) le suivi de croissance de micro-organismes extrémophiles. Ensuite, nous présenterons diverses utilisations de microréacteurs HP pour l'étude aux petites échelles des mécanismes intervenant lors des applications de stockage souterrain de déchets (CO_2) ou d'énergie (hydrogène, géothermie) : mesure de solubilité, suivi des processus de carbonatation, bioconversion du CO_2, mécanismes de bouchage de milieux poreux, etc.



Exemple de microréacteur en saphir développé à l'ICMCB.

Recent Trends in Microfluidics
workshop @ ENSMAC, Talence, 10 June 2024

Control of colloidal crystallization under confinement with light

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Crystallization refers to the process of solid formation from a fluid state. It is widely observed in nature, for instance with atmospheric ice formation. The heterogeneous nucleation of crystals relies on the use of seeds, or impurities, to favor the growth of crystal and reduce nucleation timescales. It offers a promising route to control the growth and crystalline structure of materials. In our lab, we aim at investigating the role of the seed structure, shape, and size on crystallization. To this end, we study seeded crystallization using microscopic model colloids. We use optical tweezers to organize colloids into predefined clusters and trigger nucleation events inside a bath of freely diffusive colloids, following a fine control of the seed architecture. We further confine our colloids into geometrical boundaries, made by soft lithography, to investigate the competing effect of the boundaries against the seed growth. Our work has potential to open novel routes for rational programming of the architecture of a material, from the bottom-up.

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Titre : Développement d'une puce microfluidique de PCR quantitative pour suivre la dynamique d'une communauté synthétique de microorganismes ayant un potentiel effet de biocontrôle contre le mildiou de la vigne

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Texte en Times New Roman, Taille de police : 11, interligne continu, comportant au maximum trois références bibliographiques.[1]

Le mildiou de la vigne, causé par l'oomycète *Plasmopara viticola*, est responsable chaque année d'importantes pertes économiques, pouvant entrainer des pertes de rendement allant jusqu'à 90% (Toffolatti et al., 2018). Dans l'objectif de limiter l'utilisation de pesticides, incompatibles avec le développement d'une viticulture durable mais encore très largement utilisés, des solutions de biocontrôle telles que la création de communautés synthétiques de microorganismes émergent peu à peu (Marín et al., 2021). Dans ce travail, une communauté synthétique de microorganismes ayant un potentiel effet de biocontrôle contre le mildiou a été développée. Cette communauté comporte 42 espèces microbiennes, dont 17 bactéries, 19 levures et 6 champignons filamenteux. Afin de suivre l'établissement de la communauté sur les feuilles de vigne, une puce microfluidique de PCR quantitative a été mise au point. La microfluidique offre ici l'avantage d'effectuer un grand nombre de réactions rapidement et à moindre coût en comparaison avec un système de PCR quantitative classique (Kleyer et al., 2017).

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Recent Trends in Microfluidics
workshop @ ENSMAC, Talence, 10 June 2024

High-throughput antimicrobial peptide screening using droplet microfluidics

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The threat of bacteria resistant to multiple antibiotics is a major public health issue. While infectious pathogens are becoming increasingly resistant to the drugs available on the market, the antibiotic discovery programs of major pharmaceutical companies have struggled to produce new classes of antibiotics for the past 40 years. To counter resistant bacteria, new strategies must therefore be sought to develop antibiotics with modes of action and resistance mechanisms different from those of existing drugs.

The bacterial ribosome is a validated target for antimicrobials, whose biological function is to translate the genetic information encoded in messenger RNA into protein.[1] It is inhibited by more than half the antibiotics in use today and developing peptide antibiotics that target the ribosome could be a promising source of new therapeutics.

Here, we use the bacterial ribosome both as a target and as a tool for high-throughput production and screening of peptides with antibacterial properties.[2] Specifically, we present a droplet microfluidics workflow to select peptides that inhibit bacterial protein synthesis from a library of $>10^5$ peptides produced in picoliter-sized water-in-oil compartments using a PURE translation system. Thanks to a temperature-controlled Peltier holder for microfluidic chips currently under development in our laboratory, we further aim to streamline our experimental workflow. Coupled with other high-throughput screening methods for bioactive peptides, our approach has the potential to identify new candidate molecules to be developed as antibiotics against multi-resistant pathogens.

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Recent Trends in Microfluidics
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Osmotically-driven flows and passive loading in artificial phloem: a microfluidic approach

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To ensure sap transport, vascular plants possess a complex network formed of two "microfluidic channels", the xylem and the phloem, coupled by biological membranes. On one hand, a water flow is induced along the xylem by evapotranspiration, which drains water from the roots up to the leaves where it evaporates. On the other hand, the sugars synthesized by photosynthesis in the leaves diffuse into the phloem and are eventually transported to the roots, new shoots and fruits, by a flow induced by the osmotic pressure between xylem and phloem. Such a complex mechanism has yet to be understood, in particular its dynamics. For instance, the effects of droughts and diurnal cycle on the transport of the sugars remain open questions to date. This work investigates osmotically-driven transport of sugars in the phloem. We present here the fabrication of a phloem-like microfluidic device able of handling osmotic pressures within the same order of magnitude as in vivo organisms (0.1-1 MPa). Two channels are coupled by integrating a hydrogel membrane permeable to water and with a low molecular weight cutoff (MWCO). One channel is connected to a constant supply in solute, ensured by passive loading of sugars. This work is dedicated to the characterization of the physical parameters of the hydrogel membrane and of the osmotically-induced flow, and the biological relevance of the microfluidic system is discussed based on the estimation of dimensionless groups [Comtet et. al., Nature Plants (2017)]. In the future, an artificial xylem reproducing evapotranspiration will be coupled to the current device by integration of other hydrogel membranes.

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3D Anderson localization of acoustic waves

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Anderson Localization is probably one of the most fascinating and remarkable wave phenomena since its theoretical prediction in 1958[1]. The experimental demonstration of this halt of diffusive transport in three-dimensional (3D) disordered systems has been the focus of intense and continuous research for several decades whether for quantum particles or classical waves. In the latter, a priori simpler case, experimental studies focused on vector (electromagnetic or elastic) waves in fairly complex solid structures. Inspired by recent advances in the field of 3D metamaterials[2] exhibiting unusual properties or exotic transport regimes, here we show that a locally resonant ultrasonic metafluid consisting of a concentrated suspension of soft metallic beads, engineered using soft-matter techniques, is the seat of 3D Anderson localization of scalar acoustic waves. By reporting a set of two independent time- and position-resolved experiments performed at ultrasonic frequencies, we evidence an unambiguous transition from diffusion to Anderson localization in the vicinity of the particle's resonances.

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Recent Trends in Microfluidics
workshop @ ENSMAC, Talence, 10 June 2024

Spectroelectrochemical Imaging in Microfluidic Electrochemical Devices: a pathway to reach the micromolar scale

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With the development of microtechnologies for energy conversion and storage, mass transfer and micromolar concentration variations need to be measured at the microscale. These advances need to be accompanied by novel imaging techniques with the capability to achieve high spatial resolution while detecting very small signal variations (less than 0.1%). Thus, in this work, a new microscopy technique is proposed based on a combination of electrochemical impedance spectroscopy (EIS) and visible imaging spectroscopy to measure the concentration fields at the micromolar scale in operando microfluidic fuel cells (MFCs). This technique exploits the EIS modulation and Fourier analysis to reduce the noise during the concentration field imaging. Along with the experimental developments, a mass transfer model in the periodic regime is derived to validate the measurements and to estimate the Tafel kinetics and mass diffusivities during the potassium permanganate reduction from only one potential measurement. The proposed imaging method and mathematical framework will be presented in this communication.

Life Cycle Assessment of Magnetite Production Using Microfluidic Devices: Moving from the Laboratory to Industrial Scale

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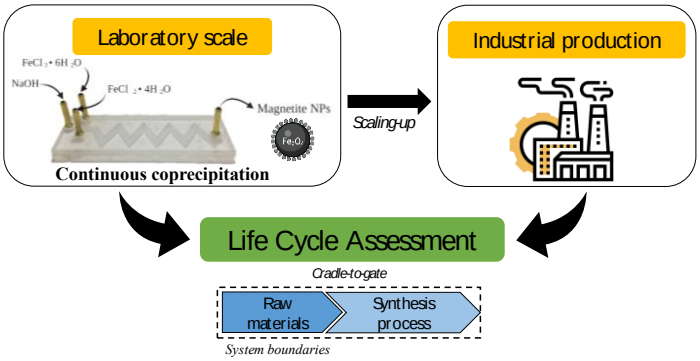

Magnetite nanoparticles (MNPs) have important applications in several industrial and scientific fields for the remediation of contaminated soil and water, for instance.[1-3] Emerging technologies such as microfluidic techniques have been adapted to continuously synthesize MNPs showing appealing results, such as a narrower size distribution.[4-6] Therefore, this approach might become important for producing MNPs to meet industrial requirements.

In this study, a life cycle assessment (LCA) is conducted to analyze and evaluate the impacts of the synthesis of MNPs performed in microfluidic devices. This LCA considers all of the steps required for MNP production at a laboratory and possible industrial scales. This work proposes a possible scaling-up of the synthesis of MNPs using many microfluidic devices working in parallel. Such an increase is envisaged to go from a laboratory scale to an industrial scale. In this context, our work aims at investigating what would be the environmental impacts of such an increase in scale and understanding the possible shifting of burdens among environmental impact categories and from one part of the product life cycle to another. Overall, the goal is to identify the portions of the process chain that contribute most to the environmental impacts as the basis for coming up with alternatives to reduce the environmental hotspots. Therefore, we carried out an LCA study considering all of the steps related to the microfabrication of the devices and to the production of MNPs at both the laboratory and industrial scales, including materials, electricity, and wastewater generated.

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In situ microfluidic investigations of APIs crystallization dynamics in $scCO_2$: From thermodynamic equilibrium to growth kinetics

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In the intricate landscape of pharmaceutical manufacturing, crystallization stands as a fundamental unit operation, constituting approximately 90% of the isolation of active pharmaceutical ingredients (API)[1]. Supercritical fluids, notably CO_2, are gaining prominence as environmentally sustainable alternatives due to their unique properties. Furthermore, in an era of miniaturization, microfluidic technology enhances heat and mass transfer, reduces preparation volumes and waste, improves reproducibility, and, crucially, provides optical accessibility for *in situ* analyses such as optical microscopy, UV, IR, and Raman spectroscopy. This study focuses on utilizing a specialized microfluidic platform under supercritical CO_2 ($scCO_2$) conditions to crystallize APIs, enabling an in-depth understanding and real-time observation of crystallization under elevated pressures.

The development of the microfluidic platform presented a significant technical challenge, requiring the design of tailored micro-reactors. The validated design ensures precise CO_2 diffusion within a micro-well system, presenting a pioneering approach for observing and studying. Prior to crystallization, the study addresses the rapid attainment of phase equilibrium through the microfluidic system, particularly focusing on the CO_2-acetone mixture. The experimental setup and protocol detail the reliability of the microfluidic device in examining phase equilibria in binary and ternary systems under pressure. Raman spectroscopy emerges as a key *in situ* characterization tool, facilitating the quantification of CO_2 composition over time, with values validated through numerical simulations.

Experimental efforts concentrate on the crystallization of naproxen, our model pharmaceutical compound. Parameters such as initial concentration, pressure, and stereoisomerism are explored. Results indicate that near-saturation concentrations reduce crystallization time, regardless of pressure, aligning with classical crystallization theories. The presence or absence of a counter-enantiomer significantly influences crystallization times, suggesting potential avenues for enantiomeric separation. In the context of crystal growth kinetics, the analysis of 2D images acquired via optical microscopy facilitates the estimation of growth kinetics through an inline model[2]. Unexpectedly, observations at 8 MPa demonstrate higher growth values than those at 10 MPa. The occurrence of liquid-liquid phase separation, a rare phenomenon, is observed, accompanied by theoretical thermodynamic propositions.

The microfluidic platform is also repurposed as a mini GAS (Gaseous Anti-Solvent) reactor for the rapid characterization of the S-NPX2:BiPY1 co-crystal under high pressure[3], thanks to Raman spectroscopy, showcasing its versatility.

In brief, this in-depth study not only broadens our understanding of pharmaceutical crystallization but also offers a unique perspective on the process under supercritical CO_2 conditions. The fusion of $scCO_2$ and microfluidic technology suggests a potential shift in how we produce pharmaceutical compounds, especially those with low solubility to increase it. Overall, this study stands out by highlighting the synergy between advanced technology and scientific exploration, providing profound insights into the complex dynamics of crystallization under $scCO_2$[4].

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Abstracts

Posters

Recent Trends in Microfluidics
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Explorer les mécanismes de dépollution des sols par les champignons

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Dans le but de réhabiliter des friches industrielles contaminées par des polluants organiques et plus particulièrement les hydrocarbures aromatiques polycycliques (HAP), diverses méthodes de traitement des sols sont mises en œuvre. Parmi celles-ci, la bioremédiation, en particulier la mycoremédiation, émerge comme une solution plus économique et durable que les traitements thermiques, physiques ou chimiques susceptibles de produire des co-produits potentiellement toxiques ou d'altérer les propriétés physico-chimiques et biologiques du sol. Ce type d'approche repose sur l'utilisation de champignons tel que les champignons telluriques et filamenteux dont le mycélium est extrêmement fin, de l'ordre du micromètre, permettant ainsi l'étude de leurs comportement au sein d'un système microfluidique (Richter et al., 2022). Le projet Mycoflu repose sur l'utilisation de puces microfluidiques pour concevoir des chambres de culture à façon dans lesquelles le champignon *Talaromyces helicus* est inoculé. Un milieu nutritif s'écoule à travers la puce, alimentant l'organisme en nutriments favorisant sa croissance (Baranger et al., 2020). Du sol contaminé par les HAP, notamment par le benzo[α]pyrène (B[a]P), un polluant classé CMR (Cancérogène, Mutagène et Reprotoxique) est introduit dans les microchambres, au contact des hyphes fongiques. Les microsystèmes sont analysés en microscopie afin d'observer et de comprendre les mécanismes impliqués dans l'incorporation du B[a]P qui possède une fluorescence intrinsèque, et donc dans le processus de mycoremédiation de ce polluant (Baranger et al., 2021).

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Development of an "Intestine-On-chip" to study infections by opportunistic pathogenic yeast Candida with advanced customized optical microscopy.

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Conventional *in vitro* biological models (cells in a Petri dish or Transwell inserts) fail to recapitulate the complex physio-biology of the human body. Alternatively, mouse models are now avoided not only because of ethical issues but also because of a lack of overlap between human and rodents[11]. Organs-on-chips (OOCs) are an alternative to model organ functionality and recapitulate some of their physiological or pathological features *in vitro*[2]. Even though the two-chamber commercial design of OOC is almost ideal to recapitulate the physiological conditions encountered in the intestine, its operational design intrinsically does not allow to observe real-time events under flow in culture compatible conditions. The overall objective of the project is to develop a new generation of OOCs in conditions that closely mimic the in vivo configuration, i.e. allowing the application of external mechanical cues (flow and stretching). The combination of a confocal microscopy module for high-resolution (but slow) fluorescence imaging with an Optical Coherence Tomography (OCT) module for lower (~µm) resolution but fast and label-free acquisition is envisioned. We aim to provide an in-depth investigation of the mechanisms underlying intestinal infection by *Candida* yeast with the perspective of identifying new routes for therapeutic treatments. The Intestine-on-chip consists of a microfluidic chip with 2 micro-channels separated by a central porous membrane, on either side of which epithelial cells and vascular endothelial cells will be adhered, mimicking the interface of a vascularized human organ. Two lateral vacuum channels allowing the generation of mechanical stretching of the membrane will be included to mimic *in vivo* intestinal cells environment.

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Introducing extreme microfluidics to study deep-sea vents microorganisms at lab scale

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Deep in the Earth oceans, deep-sea vents (DSVs) offer unique ecosystems that support life, without photosynthesis, on a wide range of steep physical and chemical gradients. However, they are still poorly understood, and traditional cultivation methods on solid media or in batch-reactors can be time-consuming and with technical biases. Using newly-developed micro- and millifluidics technologies will help answer questions that remain on (i) the DSVs microbial population and lifestyle, (ii) the limits of life in these environments and (iii) the impacts of extreme conditions on both habitability and life detection in these systems. In the frame of my PhD project, I study these specific environments, and representatives of archaea living within them, at lab scale using transparent, and high-pressure, microfluidics and millifluidics reactors under extreme conditions. These small-scale reactors reproduce the dynamic geochemical properties, as well as the porous confined environments of DSVs. The objectives of my PhD are: (i) to perform fast-screening phenotyping of DSVs microbial life to determine their adaptation strategies and their boundaries (while coping with high pressure, heavy metals concentrations and thermo-chemical gradients conditions) and (ii) to investigate the DSVs chimney minerals - microbial interactions, and colonization. The ultimate goal is to decipher both the microbial diversity, their dynamics within the DSVs, and their resilience strategies, in order to bring new input about DSV ecosystems biosignatures (*i.e.* biomolecules, metabolic function, cellular morphologies and biomineralization).

La PCR micro-fluidique : une technique facilitatrice dans la recherche prospective d'agents pathogènes

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Parmi les nombreuses utilisations de la PCR micro-fluidique, la détection des agents pathogènes est l'une des applications grandement facilitées. Cette technique permet de détecter un large spectre d'agents pathogènes dans un grand nombre d'échantillons comme pour l'étude menée en Corse et présentée ci-dessous.

La Corse est une île méditerranéenne française marquée par de fortes activités humaines et animales au sein de différents biotopes. C'est dans ce contexte que nous avons mis en place une étude ciblant la recherche d'agents pathogènes de trois groupes d'animaux et leurs vecteurs dans les zones humides de Corse. Un premier groupe est celui des mammifères sauvages, ayant pour modèle le sanglier. Le deuxième groupe est celui des animaux d'élevage corses. Le troisième est celui de l'avifaune sauvage. Par le biais d'un outil de PCR micro-fluidique en temps réel à haut débit (BioMarkTM dynamic arrays, Fluidigm Corporation, USA) développé par le Laboratoire de Santé Animale ANSES de Maisons-Alfort, 34 espèces et 11 genres de bactéries, parasites et virus ont été recherchés dans un total de 2214 échantillons provenant des trois groupes d'animaux cibles. Six genres d'agents pathogènes ont été détectés dans les ectoparasites et dans les animaux : trois genres bactériens (*Anaplasma*, *Ehrlichia*, *Rickettsia*), deux genres de parasites (*Babesia, Trypanosoma*) et un genre viral (Flavivirus). Parmi ces différents genres, certaines espèces ont été identifiées pour la première fois en Corse comme pour : *Rickettsia helvetica* dans les tiques de l'avifaune, *Ehrlichia chaffeensis* dans les tiques et oiseaux échantillonnés et *Anaplasma capra* dans des élevages d'ovins. En particulier, la présence d'agents pathogènes a été confirmée : des bactéries des genres *Rickettsia* dans des tiques de sangliers et bovins, et *Anaplasma* dans des élevages d'ovins et bovins, des parasites du genre *Babesia* dans les tiques de sangliers et bovins ainsi que dans les populations de sangliers, et du genre *Trypanosoma* dans les populations de bovins.

Fort de cette expérience, cette technique de PCR micro-fluidique va également être déployée en Nouvelle-Aquitaine (NA) dans le cadre du projet EMERG, financé par les Programmes Scientifiques à Grandes Ambitions Générales (PSGAR-MIE) de NA. Ce projet repose sur le concept d'exposome qui englobe l'ensemble des facteurs d'exposition à l'environnement extérieur, spécifiques et non-spécifiques auxquels l'avifaune sauvage et d'élevage est soumise, et les conséquences pathologiques de ces expositions. Au sein de cet exposome, les agents pathogènes représentent une menace majeure sur laquelle le projet EMERG porte toute son attention, pour par exemple confirmer à la fois l'apparition d'agent zoonotique à potentiel épidémique tel que l'Influenza Aviaire H5N1, la forte diversité des agents microbiens affectant l'avifaune (voir des coinfections) et également leur dispersion favorisée par la migration aviaire. Au sein de ce projet, une large variété d'agents pathogènes va donc être recherchée (bactéries, parasite, micromycètes, et

virus) dans une diversité de substrats combinant prélèvements d'oiseaux (fèces, sang, écouvillons cloacaux, plumage…) et environnementaux (aérosol, eaux, sols…). L'emploi de la PCR micro-fluidique permettra de faciliter les recherches et d'avoir des résultats couvrant la majeure partie de l'exposome microbien de la faune aviaire de NA.

Mots-clés : PCR micro-fluidique, Migration, Avifaune, Exposome,agents pathogènes.

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Super-deformability of induced pluripotent stem cell cysts

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Organoids are three-dimensional cell cultures derived from stem cells that, upon differentiation, resemble the cellular complexity of organs. They can be generated by isolation of adult stem cells or from induced pluripotent stem cells. In the latter case, when embedded in a reconstituted basement membrane, they may give rise to spherical epithelial cysts of self-organized hiPSCs. These lumen-containing structures have been shown to model morphologically and phenotypically the epiblast, which gives rise to the fetus in embryo development. Upon implantation, the epiblast undergoes significant remodeling from a rosette to a disc, which suggests significant deformability. In this work, we report direct measurement of the deformation and permeability in hiPSCs cysts. These measurements, coupled with live imaging, establish that these lumenized structures exhibit super-deformability induced by the formation of micro-fissures that do not impair permeability of the system. We have developed a "nano-inflation apparatus" similar to the one that would be used to inflate a ballon. A glass capillary is inserted into the ballon and fluid is injected inside using microfluidic pressure-driven pumps with flow control. It is observed that (1) the monolayer of cells does not collapse when it is poked with a micropipette, suggesting that the cyst is not pressurized, (2) upon inflating the lumen, the lumen volume increases from 0.5 nl to 5 nl, corresponding to an area-strain of 400 % before rupture. Surprisingly, volume conservation of the cell layer breaks down during inflation. Using extensive imaging with fluorescent polymers as markers, we observed the opening of non-through micro-fissures that allow to preserve the impermeability of cysts. We propose a simple mathematical model that quantitatively describes the underlying plasticity mechanism. Remarkably, we demonstrate, as a critical test, that (1) the mechanism holds for various cyst size and extracellular matrix used, and (2) the rupture stress is dependent on the extracellular matrix. After the cysts have been inflated and/or ruptured, no modifications in the proliferation and shape of the assembly are observed over days. Also, if the lumen is deflated by draining out the fluid contained in the lumen, the cyst structure is recovered within 1 hour without any alteration in growth. This "homeo-static" nature of the cells also probably indicates that the material properties of the assembly dominate over the plasticity of the individual cells, hence making this cellular assembly a unique living system that buffers stress at the individual scale.

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Wort Fermentation with Encapsulated Yeast to modify the Beer Flavour and Facilitate Filtration.

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Beer is one of the most popular alcoholic beverages in the world. Traditionally, freely suspended yeast is utilised to ferment the wort in a vat to produce beer. However, the precise control of the by-products produced by the yeast can be challenging. By-products such as esters are desirable as they contribute to aroma of the beer and makes the beer full bodied. On the other hand, by-products such acetaldehyde, vicinal diketones and higher alcohols influences the beer to be more grassy, buttery, and plastic solvent like respectively making them undesirable. We intend to develop a novel brewing technique utilising encapsulated yeast instead of freely suspended yeast.

The yeast is encapsulated within an alginate hydrogel shell utilising a microfluidic technique called the Cellular Capsule Technology, which was previously developed to encapsulate mammalian cells. As the yeast is encapsulated, a "softer" fermentation would occur thereby producing a higher proportion of desirable by-products compared to undesirable by-products. Moreover, as the yeast is encapsulated and therefore contained, it would also facilitate the filtration and clarification of beer.

Indian Pale Ale (IPA) was prepared with our new brewing technique. Gas chromatography–mass spectrometry (GC–MS) showed an altered chemical profile of the beer produced with encapsulated yeast compared to free yeast. Blind tasting of the beers revealed a noticeable difference between the two different fermentation techniques. Though individual tasters reported a partiality towards a particular fermentation method, no general preference for a particular fermentation method could be deduced.