## Workshop #1 (LAAS)

**3D printing for biology**
*Bastien Venzac, Julie Foncy, Laurent Malaquin, LAAS, Toulouse*

In this workshop, located inside the MultiFab platform of the LAAS-CNRS, we will propose an overview of our activities corresponding to 3D printing for biological applications. In particular, 3D printing of acrylate resins by stereolithography, extrusion printing of hydrogels, high resolution light-based printing of gelatine-methacrylate for scaffold fabrication will be shown and discussed.

## Workshop #2 (LAAS)

**Intracellular passive microrheology using genetically-encoded multimeric nanoparticles, GEMs**
*Morgan Delarue, LAAS, Toulouse*

In this workshop, we will introduce GEMs, which are genetically-encoded nanoparticles which can be used to infer rheological properties of the cells, and in particular how crowded they are. We will show how we can image them, how we can analyse the trajectories, and what kind of information can be extracted from their motion. We will show certain applications of the GEMs to investigate how their motion is affected by mechanical stress.

## Workshop #3 (LAAS)

**Parallel on-chip micropipettes enabling quantitative multiplexed characterization of cell aggregates rheology**
*Sylvain Landiech, LAAS, Toulouse*

**Short description:**
Micropipette aspiration (MPA) is one of the gold standards for quantifying biological samples' mechanical properties. However, relying on the manipulation of individual home-made glass pipettes, MPA suffers from low throughput and no automation. We will introduce the sliding insert micropipette aspiration method (SIMPA), which permits parallelization and automation, thanks to the insertion of tubular pipettes, obtained by photolithography, within microfluidic channels. We will show its application to quantify the viscoelasticity of 3D cell aggregates.
Please let me know if you need any additional information.

## Workshop #4 (LAAS)

**Maturation of cardiomyocytes using electromechanical stimulation**
*Christian Bergaud, LAAS, Toulouse*

We will introduce a method to enhance the maturation of human iPSC-derived cardiomyocytes.
Using 3D-printed devices with conductive biomimetic scaffolds, we will demonstrate how engineered cardiac tissues can be electrically and mechanically stimulated.

## Workshop#5 (LAAS)

**Atomic force microscopy automation, from microbes to mammalian cells**
*Etienne DAGUE (edague@laas.fr), Childérick SEVERAC (childerick.severac@cnrs.fr)*

***Abstract:***
Atomic Force Microscopy (AFM) is an imaging technique able to address living cells. It is also a force machine

providing critical information on the nanomechanical properties of living cells. However, the technology is limited because it is extremely time consuming. It is already a challenge to record data on 10 cells in a whole day. Due to this intrinsic limitation the technology is restricted to applications in the academic field. Even in this context, the small size of the data base investigated leads to sometimes inconsistent results in the literature.
It is therefore necessary to increase the throughput of the measurements and to propose solutions to realize force measurements on a larger number of cells in a reduced time. To achieve this goal we have developed a strategy for automating force measurements by AFM based on 2 points. I) directed immobilization of living cells according to well-defined cell arrays and ii) cell-to-cell movement and automatic AFM measurements thanks to an algorithm translated into a script executable on a commercial AFM.
Thanks to this system we are able to perform measurements on 500 to 1000 microbes in 2 to 4 hours[1] or to record data on 50 mammalian cells in 2 hours.

***Description:***
The first step of the procedure is to prepare a matrix of cells. For microorganisms (*Saccharomyces cerevisiae*) we will provide patches in micro-structured Poly-Di-Methyl-Siloxane with cavities of the size of the microbe to be immobilized. This patch and the filling method are described in 2 publications[2,3] of which we will keep separate copies available to participants.
We will then show how the automation script, available in the supplementary data of the article cited above, is called on the AFM, how it is parameterized to take into account the specifics of the cell array (cell spacing, number of cells in a row), and finally how it is executed. Participants will then be able to see the AFM tip move, and record data, automatically, from cell to cell.
In a second step, we will explain the method of directed immobilization of mammalian cells. We use fibronectin micropatterns obtained by microcontact printing. This process is described in an article[4] which we will also make available to participants. Fibronectin is deposited on glass slides according to patterns determined by the structuring of a PDMS patch. Participants will be able to practice microcontact printing which is on the principle very simple. A drop of the anchor is deposited on the microstructured patch, dried, then the patch is stamped on the surface to be functionalized.
We will have previously prepared some cell chips and we will continue the workshop by showing the differences in the implementation of the automation script. Indeed, the displacements between the cells are necessarily larger and the displacement method is based on the optical microscope stage and not on the displacement of the piezo electric ceramic of the AFM. Once the sample of cells is in place and the script is set up, participants will again see the AFM tip moving and recording data, automatically, from cell to cell.
At the end of the workshop participants will have understood and experienced our strategy for automating AFM measurements at different scales. It is based on the combination of directed immobilization of cells and automation of the movement/measurement duo.

Timeline:
- 0-15 min: General presentation of the workshop, the 2 immobilization methods, the 2 automation scripts
- 15-55 min: Participants practice the directed immobilization of S. cerevisae in the PDMS patch and learn how to configure the automation script. They observe the displacement and the measurements performed automatically
- 55-95 min: The participants practice the micro-contact printing and observe with an inverted optical microscope the obtained cell arrays. They configure the automation script and observe the displacement and the automatic recording of the data, from cell to cell.
- 95-105 min: participants retrieve the data and reflect with the organizers on the perspectives opened by the significant increase in the amount of data generated by AFM, in particular the potential of analysis by machine learning.

***Keywords:***
AFM, automation, microorganisms, mammalian cells, force spectroscopy, living cell, cell adhesion

***Category:***
Module 2: Nanoscopie, super resolution (2D, 3D), CLEM, Champ proche, ME/APP: Apprendre Par la Pratique : Ingénierie instrumentale et ingénierie des systèmes (caractérisation des instruments, méthodes de pointes, nouvelles pratiques)/AEX: Ateliers expérimentaux

# Workshop #6 (LAAS)

**Dynamic cell confinement**
*Vanessa Nunez, Institut Curie, Paris*

Hands-on activity on how to dynamically confine cells in two dimensions.
The dynamic cell confinement is based on the application of a modified glass coverslip (confining slide) onto a standard cell culture dish using a suction cup device. By modulating the pressure, confinement can be released so that the cells can be recovered for further analysis. This method is compatible with most optical microscopes and molecular biology protocols.

# Workshop #7 (LAAS)

***Force Inference from 3D Fluorescence Microscopy Images of Cells***
*Hervé Turlier, Collège de France, Paris*

In this workshop, I will introduce the fundamentals of the 3D force inference method from fluorescence microscopy images, as developed in the manuscript (Ichbiah et al., Nature Methods 2023). We will work through several examples together on the computer, and you are welcome to bring your own segmented cell images (tissue or embryos) to try applying the method.

We will conclude with a discussion on the limitations and future perspectives of force inference techniques.

# Workshop #8 (CRCT)

**Imaging the 3D cellular effects and rigidity of confinement in oncology: Application in pancreatic cancer**
D'Angelo R, Arcucci S, Therville N, Denais C, Delarue M, Thibault B, Di-Luoffo M, Guillermet-Guibert J.

**Keywords**
3D imaging, Tissue Clearing, Spheroids, Pancreatic Tumors, Shear Wave Elastography, Compressive constraints

**Context**
Intratumoural spatial distribution of mechanical constraints at tissular level and its importance to influence cancer progression are poorly understood. This was explored in murine in situ pancreatic tumours induced by *KRAS* and *TP53* mutations, using 2D Shear Wave Elastography (SWE). SWE reproducibly categorized tumours with selective mechanical parameters.
Interestingly, we found that SWE high heterogeneity in part estimates increased compressive constraints. Compressive constraints emerge from confinement.
To study the underlying mechanisms, we are modelling this heterogeneity of confinement in ex-vivo 3D cultures (spheroids) of pancreatic genetically modified cell lines generated in our lab.

**Practical workshop**
**It is composed by 2 parts : 3D in vitro model (clearing techniques) and in vivo model (SWE).**
Pancreatic cancer cells were grown in 3D at different time making different size of spheroids. Confinement is mimicked through embedding in hydrogels.
1.
We will show some tools to improve immunolabeling quality, clearing efficiency as well as the choice of the type of microscopy on the whole mount spheroids. The sample specificity (pancreatic tumour cell line known to spontaneously secrete matrix components) have to be taken into account for the clearing strategies.
We will illustrate results comparing different clearing methods and the relevance of matching Refractive Index.
2.
In order to show the distribution of mechanical constraints, we will use 2D Shear Wave Elastography (SWE) on big spheroids embedded in hydrogel, as model of in situ pancreatic cancer.
The Aixplorer SWE technology is provided by CREFRE service, in collaboration with Dr. C. Pestourie.

**References**
_Rizzuti I…. Guillermet-Guibert J,* Delarue M*. *Physical Review Letter 2020*
_Therville N., Arcucci S….Guillermet-Guibert J._Theranostics 2019*
_D'Angelo R. et al. Optimization of 3D High Content Screening (HCS) acquisitions on thick samples, Workshop 083, MiFoBio 2023
_D'Angelo et al. Samples clearing guidelines, Groupe de travail en Transparisation/RTmFm, 2023
_D'Angelo et al. Kit du débutant en Transparisation, Groupe de travail en Transparisation/RTmFm, 2019

## Workshop #9 (Faculté dentaire)

**Integrating Brillouin Spectroscopy with Digital Scanned Light Sheet Microscopy**
*Divyendu Valappil, Alexis Coullomb, and Corinne Lorenzo, Restore, UMR 5070 CNRS, UMR1301 INSERM, EFS, Univ. Paul Sabatier, Toulouse*
*Faculté de Chirurgie Dentaire, 3 Chemin des Maraîchers, 31062 Toulouse, on the 5th floor.*

Join us for a hands-on workshop exploring the integration of Brillouin spectroscopy with Digital Scanned Light Sheet Fluorescence Microscopy (DLSM), a powerful combination for advancing biological research. DLSM provides precise optical sectioning to access specific functional cellular events in three dimensions. When coupled with Brillouin spectroscopy, this approach offers valuable insights into the mechanical properties of tissues and cells, providing a comprehensive understanding of the relationship between function and mechanical environment.
This workshop will cover the principles of Brillouin spectroscopy and demonstrate how it can be used to investigate micromechanics in living tissues and cells. Participants will learn to apply these techniques to enhance their understanding of biological processes, enabling more detailed studies.

## Workshop #10 (CBI-CRCA)

**Ground reaction force measurement system for the investigation of insect locomotion**
*Vincent Fourcassié, CRCA, Toulouse & Vincent Mansard, LAAS, Toulouse*

The investigation of insect locomotion often concentrates on kinetics, the measurement of forces being more complex due to the small scales (1 to 100 µN) and dimensions involved. We present a system based on an array of flexible fluorescent pillars, capable of simultaneously measuring the forces exerted by all legs. This device offers a wide field of observation as well as good temporal (60 Hz) and force (0.5 µN) resolution. In addition, we have adopted a low-tech approach, enabling us to reduce costs and facilitate deployment of the technology.

## Workshop #11 (CBI)

**Compression Force Microscopy**
*Renaud Poincloux, Myriam Razouk, Merzouk Zidane, IPBS, Toulouse*

We will present a new method, called compressive force microscopy, which involves analyzing the forces applied by macrophages phagocytosing polyacrylamide micropillars of controlled size and stiffness. We will explain how the pillars were designed, show how deformations are measured and analyzed, using an automated procedure and finite element-based mechanical simulations.

# Workshop #12 (CBI)

**Optical tweezers in living tissue**

*Thomas Mangeat, Tatiana Merle, CBI, Toulouse*

Optical tweezers have revolutionized the study of microscopic forces, allowing precise manipulation and force measurement at the cellular and molecular scale. We will review various optical tweezer techniques for force measurement, with a focus on the unique challenges of applying these methods to living tissues. We will present a strategy that combines a custom-built platform with fluorescence imaging, using lipid droplets as probes in an in vivo measurement approach. Finally, we will demonstrate live how this method enables the precise measurement of cortical forces in Drosophila tissue, achieving a resolution of a few pN/µm.