# M2 internship & PhD subject:
# Biophysical analysis of self-organization in 3D microtissues using microfluidics

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Thanks to the novel microfluidic technology developed by our lab, we can create large numbers of independent 3D cell cultures (spheroids or organoids), manipulate them and observe them with single-cell precision [1, 2]. We now wish to understand the physical foundations that determine the way the cells organize within these 3D cultures, especially when they involve different cell types, and how the structural organization determines the biological function of the cells.

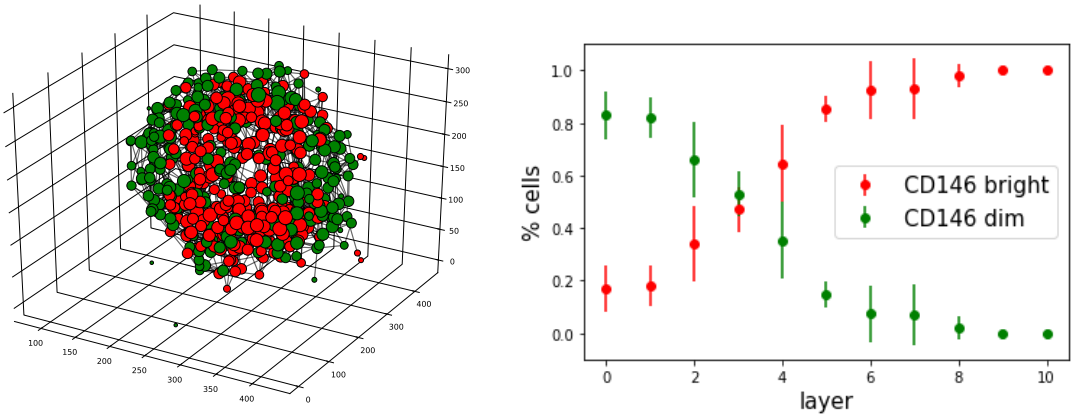


Figure 1: *(left)* Mapping of cell positions and cell-cell contacts within an organoid from a 3D confocal microscopy image. In red are CD146 $^{bright}$ cells and in green CD146 $^{dim}$ (see [3] for more details). *(right)* A connectivity graph can be used to assign each cell to a given layer, starting from the organoid edge. This shows a strong segregation between the two cell types.

We will do this by first describing of the organoid's "free energy", which accounts for the mechanical deformation energy of the cells and the adhesion energy of cell-cell contacts. This accounting will allow us to describe the 3D culture in the language of soft matter and statistical physics. For instance we wish to determine if the cells position themselves in a way to minimize this free energy, or if "active" effects keep the structure out of equilibrium. The large amounts of data that can be obtained with the microfluidic approach will allow us to quantitatively describe both the static and dynamic states of the organoids.

**Cellular models and biological questions.** We will begin by using organoids of mesenchymal stem cells (MSCs), a heterogeneous population of stromal progenitors capable of differentiating into osteogenic, chondrogenic and adipogenic lineages. We have recently shown [3] that a culture of these cells at any instant contains a mixture of states made up of undifferentiated and partially differentiated cells. We have also shown that cells organize in a hierarchical manner depending on their differentiation level when cultured in a 3D format (see Figure 1). However it is not clear what *physical* parameters determine the sorting of the cells in this manner. Beyond MSCs we will use this approach to make realistic models of solid cancerous tumours, in order to then observe the interaction between cancer and immune cells (e.g. CD8+ T-cells that attack the tumour). As such, the results can have a medical impact (e.g. cancer-immune interactions) or fundamental biophysical objective (e.g. free energy of organoids), or both.

**Multidisciplinary project and team.** This project is highly multidisciplinary, as it relies on advanced technology and physical principles to answer fundamental biology questions. It will take place at the Phyical Microfluidics and Bioengineering research unit, which is jointly affiliated with Institut Pasteur (Paris) and Ecole Polytechnique (Palaiseau). The team is currently made up of about 10 physicists, engineers, and biologists. Three of the ex-PhD students won the Ecole Polytechnique thesis award. Our research is focused on understanding the link between the single-cell characteristics and the collective properties that emerge through cellular interactions at the scale of a small population.

**The ideal candidate.** You should have a strong background in physics or engineering, with an equally strong interest and basic knowledge in biology. There is no current funding for the PhD but we will apply for fellowships together. Please send a CV, motivation letter, and the names of 2-3 persons who can recommend you to Charles Baroud: **charles.baroud@pasteur.fr**.

## References


[1] Sébastien Sart, Raphaël F.X. Tomasi, Gabriel Amselem, and Charles N. Baroud. Multiscale cytometry and regulation of 3D cell cultures on a chip. *Nat. Commun.*, 8(1), 2017.

[2] Raphaël F.X. Tomasi, Sébastien Sart, Tiphaine Champetier, and Charles N Baroud. Individual Control and Quantification of 3D Spheroids in a High-Density Microfluidic Droplet Array. *Cell Rep.*, 31:107670, 2020.

[3] Sébastien Sart, Raphaël F.X. Tomasi, Antoine Barizien, Gabriel Amselem, Ana Cumano, and Charles N. Baroud. Mapping Structure and Biological Functions within Mesenchymal Bodies using Microfluidics. *Sci. Adv.*, 6(March):eaaw7853 1–14, 2020.