PhD PROJECT PROPOSAL

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# [PROJECT TITLE] BIOMECHANICS OF THE GUT EPITHELIUM DEVELOPMENT

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**Abstract** (10 lines)*
Deciphering the dynamic cell orchestration that conditions faithful mammalian organogenesis is a critical and unresolved challenge. In this study, we focus on the mammalian gut—a highly organized monolayer epithelium where adult tissue self-renewal is driven by stem cell divisions within a specialized niche (Saleh et al., *Developmental Cell*, 2023). Biomechanics may play a pivotal role in coordinating cellular activities, thereby maintaining the integrity and architecture of the epithelium. To gain a comprehensive understanding of gut epithelial dynamics, we advocate for a multiscale, integrative approach that combines high-resolution live imaging with advanced analytical techniques. We aim to: 1) identify key events driving the dynamics of the gut's epithelium by integrating *in vivo* and *ex vivo* advanced imaging, biomechanical analysis, and physical modeling, and 2) experimentally perturb the system to uncover the cellular, tissue, and molecular mechanisms that regulates gut epithelial dynamics. This approach will yield valuable insights into the intricate processes that govern tissue homeostasis and development in the gut, deepening our understanding of epithelial biology and its implications for health and disease.

**Keywords***
Tissue morphogenesis, gut, mouse, organoids, biophysical analyses, advanced optical techniques, smart microscopy, 3D imaging

**Scientific question and Objectives** (10 lines)*
Epithelial tissue mechanics is vital for organogenesis and homeostasis. Tissue forces influencing cell shape and behavior can be intrinsic, from actomyosin activity and junctions, or extrinsic, from the surrounding extracellular matrix. Research on gut morphogenesis has primarily focused on intestinal stem cell fate and proliferation rates. However, the exact cellular and molecular events underlying crypt-villus axis emergence and maintenance are poorly understood, with limited literature on the topic. Thus, the intestinal morphogenesis is still underexplored and its limited understanding hinders insights into the participation of gut biomechanics in human disorders such as cancer and rare developmental diseases.

Building on technological developments and initial data, we aim to decipher the understudied intestinal morphogenesis Our working hypothesis is that a mechanical coupling between cellular behaviour and its environment regulates the homeostasis and architecture of the gut tissue. The objectives of this research proposal

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will thus be focused on the following key questions: What are the key morphogenetic steps occurring during gut epithelium establishment? What are the molecular and biophysical mechanisms in charge?

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**Proposed approach (experimental / theoretical / computational) and research plan** (20 lines)*

The PhD project will utilize diverse models: 1) *in vivo*: we will analyze mouse samples at various developmental stages, and 2) *in vitro*, we will use organoid cultures that enable genetic manipulations and high-resolution imaging to study tissue dynamics (Saleh et al., *Developmental Cell*, 2023; Barai et al., *BioRXiv* 2024). These cultures, grown in 3D hydrogel, will help visualizing tissue remodeling and characterize its mechanics in conditions resembling *in vivo* environments. All required mouse models and organoid lines are available in the supervisor#1's team. To live image crypt fission, we will adapt imaging set ups recently developed for drosophila embryogenesis study (Abouakil et al., *Light: science and applications* 2021, Rigato et al., *PLos Biol* 2024, Mazzella et al., *Light, science and applications* 2024). More precisely, we will combine spinning disc confocal with advanced microscopy techniques to illuminate small sample volumes, ideal for fragile organoids, and autonomously track/reposition areas of interest during 48-60h imaging sessions, increasing frequency when morphogenetic events are detected. Non-linear imaging techniques like coherent Raman microscopy and second harmonic generation (SHG) will enable label-free imaging of nuclei, and collagen in newborn mouse tissues and organoids. Polarimetry imaging and random illumination microscopy (RIM) will allow 3D imaging of cytoskeletal changes, while advanced laser ablation system combined to a smart-scanning apparatus facilitate precise biomechanical studies of tension patterns in the gut tissue. These imaging approaches are available in the supervisor#2's team. No major risk is associated with this project since the required expertise for 1) mouse work, 2) genetic manipulation of organoids, 2) imaging solutions or 3) image analyses are mastered by both supervisors' labs. Published and unpublished results recently obtained by both supervisors provide solid foundations for the PhD project.

**Interdisciplinarity and Implication of the two labs** (15 lines)*

(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

Here, we propose an interdisciplinary PhD project to reveal morphogenetic and biomechanical mechanisms that drive gut organogenesis. Through this project, we combine our expertise and technical skills to develop knowledge in developmental biology and epithelial tissues around intestinal morphogenesis, and our approach will provide tools to overcome important technical hurdles, such as the use and the imaging of intestinal organoids with high spatio-temporal resolution. The added-value of the project relies on the consortium which combines:

- a developmental biologist educated to cellular biophysics approaches (Supervisor#1) in Institut de Biologie du Développement de Marseille (IBDM), an institute dedicated to research oriented towards the understanding of how an embryo develops in adult organism and how it is maintained.
- and a biophysicist educated to epithelial morphogenesis with strong expertise in 3D imaging (Supervisor#2) in Institut Fresnel Marseille, an institute dedicated to research and innovation in optics, electromagnetism and photonics.

**Specify with whom the person recruited will collaborate and on what aspects ***

In supervisor#1's lab, the PhD candidate will directly interact with a postdoctoral researcher with strong expertise of the gut epithelium morphogenesis, organoid culture expertise and biophysical analyses (Saleh et al., *Developmental Cell* 2023; Barai and Delacour, *Methods in Molecular Biology* 2025; Barai et al., *BioRXiv* 2024), an engineer with mouse breeding and advanced histology expertise, a technician with histology and organoid culture expertise.

In supervisor#2's lab, the PhD candidate will have strong interactions with a PhD student (3rd year in October 2025) specialists in biophotonics and 3D imaging of tissues, one research engineer for the development of microscopy-compatible cell culture optimization and a postdoctoral researcher (biologist, to be recruited early 2025) who will be a user of the same imaging setups.

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**PhD student's expected profile***

The project blends different aspects. We seek a student with a will to delve in the implementation of novel experimental tools for tissue mechanics and who will enjoy performing experiments and developing image analyses. Programming skills will be an asset.

**Is this project the continuation of an existing project or an entirely new one?** Entirely new one

~~**In the case of an existing project, please explain the links between the two projects (5 lines)***~~

**Two to five references related to the project***


Guevara-Garcia A, Soleilhac M, Minc N and Delacour D. regulation and functions of cell division in the intestinal tissue. *Seminars in Cell and Developmental Biology* (2023), 150-151:3-14.

Kretzschmar K and Clevers H. Modeling development and the stem cell niche in a dish. *Developmental Cell* (2016), 38(6):590-600.

Garreta E, Kamm R.D, Chuva de Sousa Lopes S.M, Lancaster M.A, Weiss R, Trepat X, Hyun I and Montserrat N. Rethinking organoid technology through bioengineering. *Nature Materials* (2021), 20(2):145-155.

Abouakil, Meng, Burcklen, Rigneault, Galland, **LeGoff** (2021). An adaptive microscope for the imaging of biological surfaces. Light: science and applications 10, 210 (2021). DOI: 10.1038/s41377-021-00649-9


**Two main publications from each PI over the last 5 years***

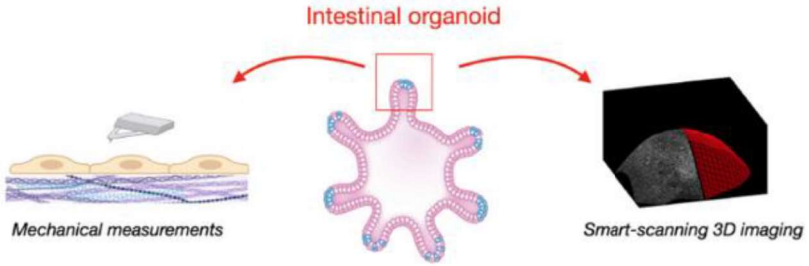

Barai A, Soleilhac M, Xi W, Lin S.Z., Karnat M, Bazeliières E, Richelme S, Berrebi D, Ruemmele F, Théry M, Rupprecht J.F. and **Delacour D**. Multicellular actin star network underpins epithelial organization and connectivity. *Under revision. BioRXiv* 2024, doi: *https://doi.org/10.1101/2024.07.26.605277.*

Saleh J, Fardin MA, Barai A, Soleilhac M, Frenoy O, Gaston C, Ciu H, Dang T, Gaudin N, Vincent A, Minc N* and **Delacour D*.** Length-limitation of astral microtubules orients cell divisions in intestinal crypts. *Developmental Cell* (2023), 58(17):1519-1533.

Mazzella, Mangeat, Giroussens, Rogez, Li, Creff, Saadaoui, Martins, Labouesse, Idier, Galland, Allain, Sentenac, **LeGoff** (2024). Extended-depth of field random illumination microscopy, EDF-RIM, provides super-resolved projective imaging. Light, science and applications DOI: 10.1038/s41377-024-01612-0.

Rigato, Meng, Charles, Runion, Abouakil, Smith, **LeGoff** (2024). A mechanical transition from tension to buckling underlies the jigsaw puzzle shape morphogenesis of histoblasts in the *Drosophila* epidermis. *PLos Biol*, https://doi.org/10.1371/journal.pbio.3002662


**Project's illustrating image**

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