M2 Internship :
Magnetic nanoprobes to image the mechanical properties of cells using digital holography

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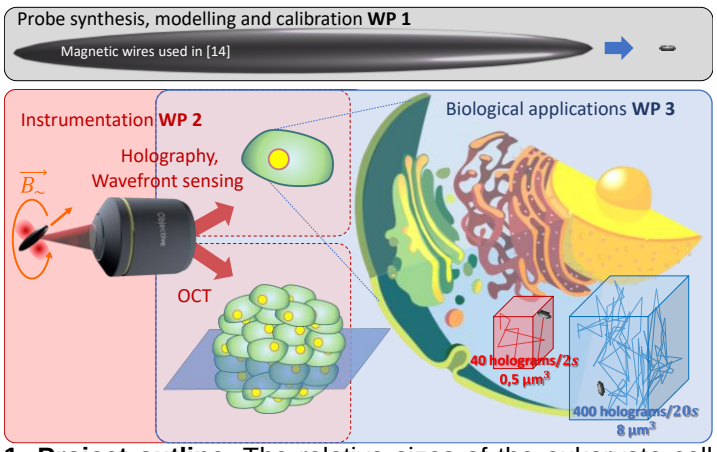
Position of the project as it relates to the state of the art

It is now well established in the fields of life sciences and biology that cells respond to active forces and changes in their rheological environment by turning mechanical stimuli into biochemical signals[1].



1- Project outline. The relative sizes of the eukaryote cell (right, 10 µm - source :Wikipedia), viscosity probes (10 µm maghemite wire and 200 nm maghemite nanorods), and experimental trajectories are drawn to scale.

Mechanonsensing is just as essential as chemical sensing and biochemical signal pathways in the regulation of cell growth, proliferation, migration, differentiation or apoptosis, and in the regulation of developmental processes and tissues homeostasis. This field of studies, initiated by biophysicists, is now reaching out to other fields, including materials science and chemistry (e.g. polymers, soft and complex matter), clinical research (mechanical properties are crucial to cancer cells) and physics to model these phenomena and develop new tools for their investigation. Mechanosensing also depends on the intracellular rheology, which is heterogeneous and actively responds to the extracellular mechanical properties [2-5]. A quantitative and reliable determination of the rheological parameter of intracellular compartments is indispensable to understand the development and homeostasis of living tissues and organs. **The multi-scale approach proposed here aims at developing and disseminating instruments able to improve the spatial resolution of viscosity/elasticity mapping by one order of magnitude, able to operate in single cells as well as cell aggregates and organoids.**

We have recently shown [15] that heterodyne holography is an appropriate tool to analyse the **rotation of magnetic nanorods**, a concept borrowed from macroscopic rheology. By improving the sensitivity, holography allows a **reduction of the rod size by 1 order of magnitude**, which paves the way for **ultralocal mechanical measurements** and a multi-scale characterization of the microscopic origin of cell mechanical properties. In this project, we will use 3D superlocalization to track nanoprobes during their Brownian exploration of the environment.

1. Instrumental development

resolution improvement . We have shown that 3D superlocalization allows nm-range precision[23,24] of nanoparticles as small as 30 nm, and that stochastic movements of the particle can be used to explore the environment and obtain a dense 3D super-resolved map (ANR 3DBROM)[25,26]. Here, we will reduce the probe size down to **100-200 nm** (WP1) but also, crucially, the acquisition time τ_{acq}. Indeed, during the measurement, Brownian probes travel stochastically over $\sqrt{D\tau_{acq}}$ (where D is the viscosity-dependent diffusion coefficient)[27]. This diffusion (see experimental trajectories in fig.1) affects the locality of the measurement. Preliminary

experiments have shown that holographic detection can reduce τ_{acq}, allowing to probe a volume of (0.5 μm^3) (see fig 1), and can be pushed further by increasing the magnetic field, frequency, and optical intensity.
The method a **clear dissemination/industrial potential**, and we will pay particular attention to intellectual property protection (possible patents).

2. Applications

These approaches will be applied to quantitatively map elasticity and viscosity at the subcellular scale on well-defined 2D cellular models for which our collaborators at Institut Jacques Monod have a strong experience.
These methods will be applied to **2D cellular models**, in which the viscoelastic properties depend on the mechanical coupling of their cytoskeleton composed of actin filaments, microtubules and intermediate filaments, with their environment[35]. The IJM team has developed MDCK cells silenced for Myosin isoforms, which are strongly affected in their acto-myosin organization[29]. The viscosity and elasticity parameters of these cells will be mapped and compared with control (wild type) cells.
While palpation detects solid breast tumours because of their stiffening compared to the surrounding tissue, cancer cells tend to soften. Rigid tumor observations actually reveal that the number of softer cells increases with the invasiveness**. Tumoral 3D spheroids** recapitulate tumour morphology and global organization, and display altered mechanical properties. Exploring tumoral spheroids and their mechanical heterogeneities by measuring viscosity and elasticity is of tremendous importance in the understanding of tumoral invasion.

The **M2 intern** will mostly contribute to the development of the holographic microscope (part 1). Most of the effort during the internship will be devoted to Part 1 (instrumental development). Applications to biology (part 2) will be adapted to the wishes and skills of the intern. He/she should have a good knowledge of optics, an interest for experimental development and programming. Although the applications are mostly biology-oriented, no particular biological knowledge is necessary.

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As such, the internship as well as the following PhD are already funded.

- References related to the project References from consortium members in bold.

[1] S. Suresh, Acta Biomater., 3 (2007) 413
[2] **W. Xi, et al., Nat. Rev. Mater., 4 (2019) 23**
[3] **Doss BL, et al. PNAS. 2020 117(23):12817**
[4] **Sarangi BR, et al. Nano Lett. 2017 17(1):399**
[5] **Gupta M et al. Nat Comm. 2015 6:7525**
[6] **Ladoux B, et al. Nat Rev Mol Cell Biol. 2017 18:743**
[7] G. Bao, et al., Nat. Mater., 2 (2003) 715-725.
[8] T.M. Squires et al. Ann. Rev.Fluid Mech. 42 (2010) 413
[9] E. Moeendarbary, et al., Nat. Mater., 12 (2013) 253
[10] A.R. Bausch, et al. Biophys. J., 75 (1998) 2038
[11] B. Fabry, et al., Phys. Rev. Lett., 87 (2001) 148102.
[12] D. Mitrossilis, et al. PNAS., 107 (2010) 16518
[13] F. Etoc, et al. Nat. Mater. 17 (2018) 740
[14] **J. Fresnais, et al. Adv. Mater., 20 (2008) 3877**
[15] **C. Gentner, et al. ACS Nano (2024)**
[23] **M.C. Nguyen et al. ACS Nano, 2c04792 (2022)**
[24] **V. Brasiliense et al. ACS Nano, 12 (11), 10833 (2018)**
[25] **A Martinez et al. Opt. Ex.22, 23, 29191 (2014)**
[26] **J.-F. Rupprecht et al., Opt.Exp. 25(19) 23146 (2017)**
[27] **C.Gentner et al., Opt. Exp. 29, 1, 527 (2021).**
[28] **T.Wu et al. Opt.Lett. 47, 12, 3079 (2022)**
[29] **P Xiao et al., Optica 5 (4), 409-412 (2018)**
[30] **J Scholler et al. Light: Sci. & App. 9 (1), 140 (2020)**
[31] M.Heuzé et al. Elife 8 (2019): e46599.
[32] **S. Sonam et al. Nature physics, 19(1), 132 (2023)**
[33] **Balasubramaniam, et al. Nature Mat., 20(8) (2021)**
[34] **Saw, T. et al., Nature** 544**, 212–216 (2017).**
[35] E.Infante et al. Front Cell Dev.Bio. 10 (2022): 951816
[36] **I.Nagle et al. Front. Cell Dev. Biol 10 (2022) 10.3389**