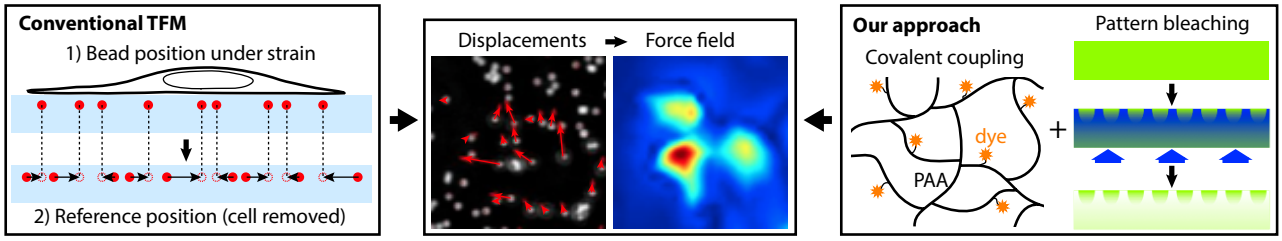
High-resolution traction force microscopy

Mechanical forces exerted by and upon biological samples have recently emerged as a critical aspect of cell fate, morphogenesis and tumor growth. It is now widely admitted that **cells are sensitive to their mechanical environment** and that sensing occurs through active processes such as attachment and pulling on the surrounding substrate. In this context, **the development of methods to measure forces exerted by cells has drawn considerable interest, in particular traction force microscopy** (TFM). TFM has been successfully used to map forces created by eukaryotic cells, which typically spread over several hundreds of μm^2 and exert local forces in the 1-100 nN range. In contrast, studies reporting force quantification and mapping for bacteria are extremely scarce. This is due to the small cell size (a few µm) and low forces (~100pN) generated, which require pushing further the sensitivity limit of existing methods.

The aim of this project is thus to develop high-resolution traction force microscopy and to apply it to study forces exerted by individual bacteria.

Most TFM implementations use a flat, soft film of hydrogel of tunable elastic properties, seeded with nanobeads whose motion is tracked optically to monitor the surface displacements induced by cell-generated forces. State-of-the-art high-resolution TFM techniques provide ~1µm resolution. A major limitation of this method is that irregular seeding does not provide optimal sampling of the displacement field. In addition, poor bead/matrix coupling for the smallest beads results in irreversible motions relative to the surrounding polymer mesh, and hence increases the noise level of force measurements.



Here, we propose instead to use **optical patterning of homogenous fluorescent gels** to achieve sub-µm, high sensitivity TFM: Instead of randomly distributed beads, we propose to use a gel containing covalently coupled dyes that cannot diffuse through the network. Prior to cell seeding, the fluorescence is modulated by bleaching with patterned illumination to provide a diffraction-limited regular pattern whose deformation can be subsequently imaged. This approach provides a mechanically homogenous substrate and optimal, regular sampling of the deformation field that simplifies force field computation and improves the resulting spatial resolution (~500nm).

Practical implementation will tackle the following issues:

- **Obtaining PAA gels with attached dyes** using commercially available molecules.
- **Optimizing the patterning process** by testing various motives, sizes and dyes.
- **Benchmarking the force field measurements against readily existing methods**.
- **Mapping forces exerted by individual bacteria adhering on soft substrates**. Our team already has experience in TFM on bacteria, and this development will be integrated in a wider project dealing with the impact of mechanical constraint on bacterial contamination of surfaces.

For whom? This project includes microscopy, simple chemistry, image analysis and microbiology and we are thus looking for candidates eager to work in an interdisciplinary environment. Some knowledge in image analysis is a plus.

Where? The Laboratory for Interdisciplinary Physics in Grenoble brings together biophysics, soft matter physics, optics, physico-chemistry and biology in a international environment.

With whom? Delphine Débarre (microscopy, data analysis), Sigolène Lecuyer (microbiology, mecanobiology), Lionel Bureau (physico-chemistry).

Contact: delphine.debarre@univ-grenoble-alpes.fr