Imaging flowing Soft Matter *in situ*

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1. INTRODUCTION

Most systems in biology or in soft matter are extremely sensitive to the sample environment. This is why they can behave very differently under external physical or mechanical constraints. We are interested in understanding the behavior of such systems under flow, tensile solicitation, and gravity.

Confocal scanning microscopy is a powerful technique for 3D imaging and can be used to measure the spatial organization of micrometric objects with excellent resolution. This is why we have developed special environmental sample holders, coupling observation and solicitation for our microscope, Leica SP8. One of them allows to follow the creation of a biofilm of bacteria under flow and mechanical solicitation. The other is designed to study structuration in the vertical direction, so gravity dependent systems.

2. MICROFLUIDIC CHAMBER COMBINING CONFOCAL IMAGERY AND MECHANICAL SOLLICITATION TO STUDY

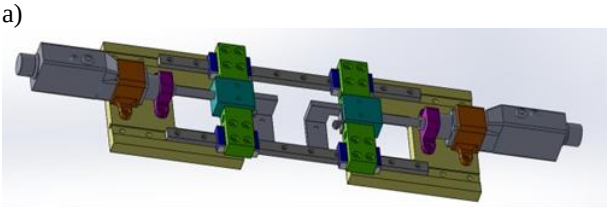
The first device is used for studying biofilms, and it combines a microfluidic chamber and uniaxial tensile device, see Figure 1a for a photograph and a scheme. The aim is to understand how bacteria can form a biofilm when subjected to a liquid flow and when the colonized surface is under cyclic mechanical solicitation.

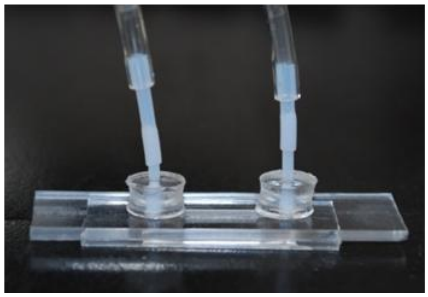
Bacterial biofilms most often are to communities that self-assemble into a cohesive extracellular matrix on solid surfaces or as pellicles floating on top of liquids. This matrix gives biofilms a high resistance to environmental stresses.

The device is composed of two paired linear actuators, each providing a displacement of 1-2 mm with a speed of 0.1 mm/s. In between the clamps, is placed a channel, 200 µm width, 20 mm length, 2 mm large, composed of 2 PDMS films. The applied flow on the channel full of bacteria is imposed by the bacteria speed, 500 µL/min.

We study a fluorescent bacteria and we can see in fig 1.b) bacteria traveling through the channel, and in very bright green color a biofilm created by the bacteria against the constraints.

a)





b)

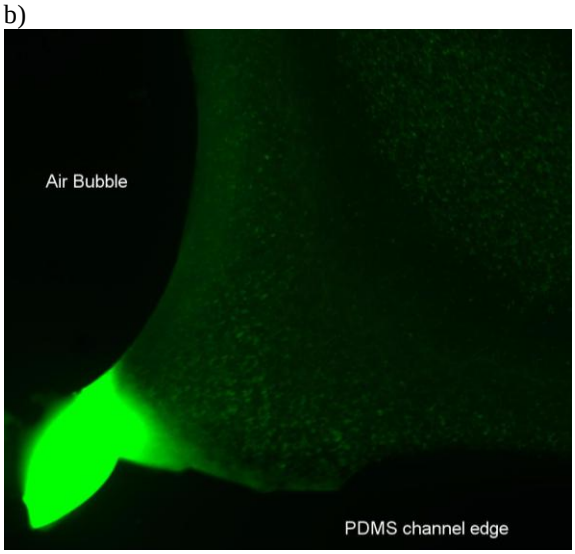


Figure 1 a) The uniaxial tensile device (on the top), and the microfluidic channel; b) Image of bacteria and a biofilm in the channel

3. FLIPPING THE CONFOCAL TO IMAGE VERTICAL STRUCTURES

Emulsions are mixtures of two immiscible fluids, such as oil and water, dispersed into droplets. If the density of the two phases is different and the drops are sufficiently large they will separate to the top (creaming) or the bottom (sedimentation) of the sample depending on whether the drops are more or less dense than the solvent. We are interested in studying the creaming process and the particle organisation when the droplets are confined inside small capillaries. We expect to see an influence of the degree of confinement on the creaming or sedimentation behavior.

Thanks to a periscope and a home-made device allowing control in 3D, we can thus study the effect of gravity on the emulsions. Indeed, the laser beam is then redirected and focused horizontally on the sample. The sample holder allows for the 3D exploration of the small capillaries, in which we study the emulsions, see Figure 2a for an example. The droplets have a diameter of tens of micrometers. A drawing of the device is shown in Figure 2.b). The periscope (LMSTech) inverts the laser beam in a horizontal way; the xy positioning is done by a motorized stage, and the focal imaging, i.e. the Z stacking is provided by a piezo system placed on the objective. The sampler holder is a capillary where the emulsion is placed.

a) b)

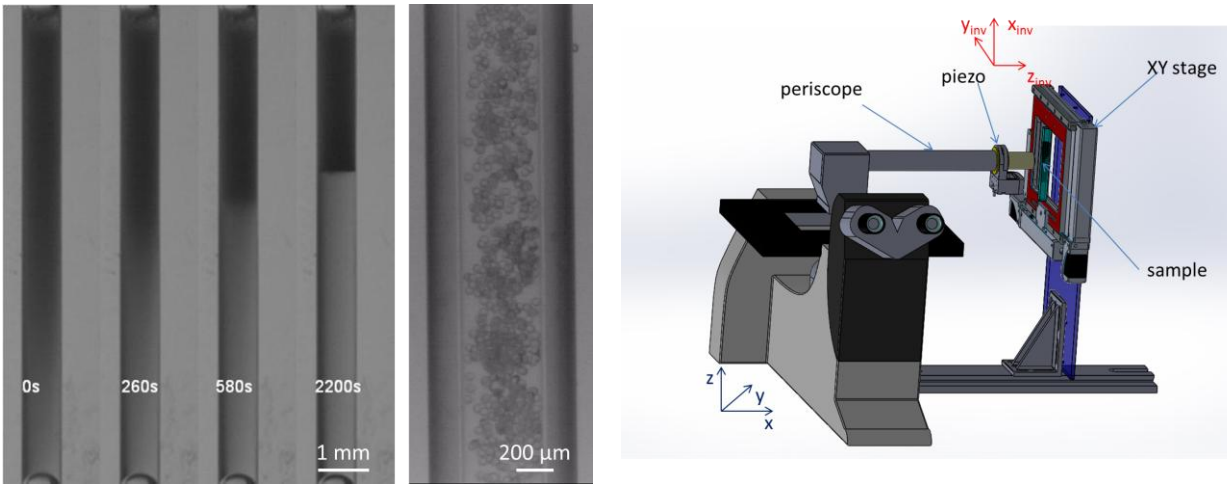


Figure 2.a) A photograph of an emulsion creaming in a capillary and a closer look at the organization of particles inside a small capillary during sedimentation, b) Schema of the 'inverted' device.

3. CONCLUSION

The confocal scanning microscopy is a powerful tool, allowing the 3D imaging in situ, when equipped with home-made environmental holders. We have described two such holders, which allow the imaging of the formation of a bacterial biofilm under external constraints and the creaming of emulsion droplets in confining vertical capillaries. These examples can give important intakes in understanding the behavior of the real systems under flow or stress.