Motility fractionation of bacteria by centrifugation

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Flagellar motility plays a fundamental biological role in prokaryotic and eukaryotic unicellular organisms. Modulating flagellar activity in response to a variety of chemical and physical stimuli, single-celled micro-organisms can effectively search for optimal environmental conditions. Flagellar motility also plays an important role in medicine, being a major contributing factor to pathogenicity and colonization in bacteria like *Vibrio cholerae*. More recently, the possibility of exploiting swimming micro-organisms as actuators for microstructures has extended the interest for flagellar motility to the physical domain of micro-engineering applications[1,2]. Recognizing the primary role of motility has led to the development of new tools that are capable of a precise and quick characterization of the dynamical properties of cells. Image correlation techniques, as dynamic image correlation spectroscopy (ICS) and differential dynamic microscopy (DDM), are promising tool offering a high-throughput method for characterizing the motility of microorganisms[3].

However, in conjunction to physical tools for motility quantification, it is also desirable to develop physical techniques for sorting colonies, that usually display a high motility variation, into spatially separated fractions characterized by a motility gradient. Fractionation by centrifugation is a widely used technique in biology and chemistry. It relies on the strong sensitivity of sedimentation speed on particle size and composition and therefore allows to separate components according to size, mass or density. As a result a stationary state is eventually reached where, each solute is distributed according to the Boltzmann law: $\rho\,(z)\ \propto \exp[-v_d z/D]$, with $v_d = \mu\Delta m a$ is the drift speed induced by a uniform centrifugal acceleration a on a particle having a buoyant mass Δm, mobility μ and a diffusion coefficient $D\ =\ \mu k_B T$. Although bacteria will display some variations in the buoyancy mass, we do not expect it to be strongly correlated to their motility. On the other hand swimming bacteria do not rely on thermal agitation for motion, but have their own source of propelling power that makes them a strongly out of equilibrium system. It has been found that in many respects, they can be thought of as "hot colloids", with an effective diffusivity that strongly depends on motility and that is typically hundreds of times larger than the Brownian diffusivity of non-motile cells[4].

In this talk we experimentally demonstrate[5] that a sample of motile *E. coli* bacteria, displaying a broad spectrum of swimming speeds, behaves like a mixture of "hot" colloids having a corresponding broad spectrum of effective temperatures and therefore sedimentation lengths (see Fig. 1). As a consequence, after centrifugation, non-motile bacteria will accumulate to the bottom of the cell while higher regions are populated with bacteria having an increasing motility/temperature. We used ICS to perform space-resolved motility measurements of bacteria observed over a field of view spanning 1 mm. Space dependent motility distribution were retrieved for centrifugal fields in the range $\sim$ 4-12 g and accounted for with a simple theoretical model of active diffusion.

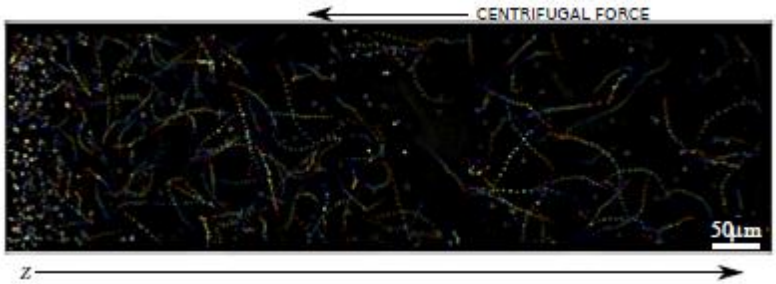

FIG. 1: Traces of swimming bacteria after centrifugation obtained from digital-videomicroscopy. Slow bacteria are found sedimented at the bottom of the sample (left-hand side) while faster ones populate the high-z region of the sample. Traces are obtained as a superposition of frames that have been colored progressively from red to blue as time increases.

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