École Polytechnique

Diffusion and clustering of passive particles in a bath of micro-algae

by Yiming Wei

Lab Rotation Report

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Acknowledgments

I am deeply indebted to Dr. Julien Bouvard for his invaluable mentorship and guidance throughout my internship. His generosity in sharing his profound knowledge and expertise has been instrumental in my learning journey. The opportunity to attend the insightful lectures of Florencio Balboa-Usabiaga and Sujit Datta, facilitated by Julien, served as my initial foray into this fascinating field. His patient instruction in the analysis of Mean Square Displacement (MSD), the privilege of accessing his comprehensive PhD thesis, and his keen eye in identifying and rectifying issues in my experiments have all been pivotal to my progress.

My heartfelt gratitude extends to Mohamed Taha Laroussi, whose hands-on teaching approach significantly enhanced my practical skills. His step-by-step guidance on conducting experiments, cultivating bacteria, and fabricating devices has been a cornerstone of my experimental work.

This internship has been a journey of exploration and discovery, made less daunting and more enriching by the unwavering support of these remarkable individuals. Their encouragement has not only equipped me with a solid foundation in this new area but also ignited my curiosity to delve deeper and continue my exploration. I am truly grateful for their contributions to my professional and personal growth.

Palaiseau, June 28, 2023 Yiming Wei

Abstract

This report explores the behavior of microorganisms, primarily the algae *C. reinhardtii*, focusing on the intricacies of their motion and interactions, both individually and in colonies. Distinguished as "pullers," *C. reinhardtii*'s flagella-driven swimming mechanism is contrasted with that of "pushers," like *E. coli*. The study investigates collective movements using the Vicsek model, an effective tool in the understanding of collective dynamics in biological systems, such as fish schools, bird flocks, and bacterial colonies. The report elucidates the experimental procedures for cellular cultures preparation and trajectory reconstruction. Finally, the diffusion behavior of these microorganisms is examined, yielding insights into different diffusive regimes based on Mean Squared Displacement (MSD) analysis.

Résumé

Ce rapport explore le comportement des micro-organismes, principalement l'algue *C. reinhardtii*, en se concentrant sur les subtilités de leurs mouvements et de leurs interactions, à la fois individuellement et en colonies. Le mécanisme de natation de *C. reinhardtii*, que l'on qualifie de "pullers", est opposé à celui des "pushers", comme *E. coli*. L'étude examine les mouvements collectifs à l'aide du modèle de Vicsek, un outil efficace pour comprendre la dynamique collective dans les systèmes biologiques, tels que les bancs de poissons, les volées d'oiseaux et les colonies bactériennes. Le rapport élucide les procédures expérimentales pour la préparation des cultures cellulaires et la reconstruction des trajectoires. Enfin, le comportement de diffusion de ces micro-organismes est examiné, ce qui permet de comprendre les différents régimes diffusifs basés sur l'analyse du déplacement quadratique moyen (DMS).

Contents

Chapter 1

Introduction

1.1 Laboratory Rotations

This report is the culmination of my one-month internship journey in the field of Biomedical Engineering (BME), a period of immersive learning and practical application. As part of the program's unique design, students are required to complete rotations in at least two different BME laboratories, spending four weeks in each. This immersive process allowed me to shadow doctoral students or postdoctoral fellows and contribute to research projects of limited scope.

1.2 The Project

The project I am involved in is primarily based on Julien Bouvard's paper titled "Ostwald-like ripening in the two-dimensional clustering of passive particles induced by swimming bacteria"[1]. This seminal work primarily describes the intriguing phenomenon of passive bead clustering(like figure:1.1) in the presence of swimming bacteria, following an Ostwald ripening-like dynamics.

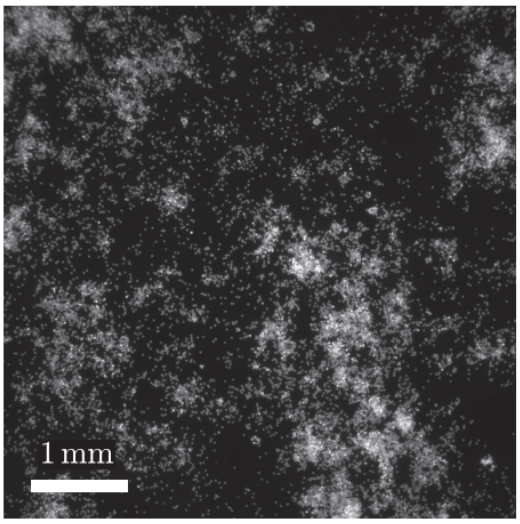

Figure 1.1: Giant bead clustering after 16 hours, obtained for beads of diameter DB = 5 μm and initial bacterial concentration of OD = 5 and surface fraction Φ_B = 0.6. The clusters themselves are organized in structures of an even larger size, up to 2-3 mm. From Bouvard *et al.* [1].

In this process, larger particles grow at the expense of smaller ones, leading to an increase in the average size of particles over time. Interestingly, the process does not appear to reach saturation, implying that the clusters continue to grow without an apparent limit[2]. The paper demonstrates that very large clusters can be formed, suggesting the potential to use bacterial motility to generate large structures of passive colloidal particles.

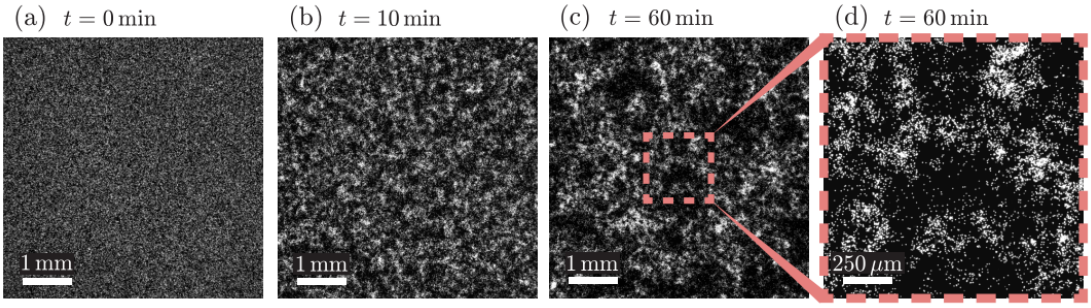

Figure 1.2: Image sequence showing the clustering of beads of diameter DB = 5 µm and surface fraction Φ_B = 0.12 induced by the activity of the bacterial bath at bacterial concentration OD = 5 (about 105 beads present on the images). Image (d) is a zoom of image (c), showing freely diffusing beads in the depleted regions between clusters. From Bouvard *et al.* [1].

These findings have significant implications for fields such as materials science or medicine, where the controlled assembly of particles into larger structures is often desirable[3], [4]. The insights from this paper form the foundation of our current project, and we aim to further explore and build upon these findings.

First, we will change *Burkholderia contaminans* to micro-algae *Chlamydomonas reinhardtii*(like figure:1.3), and will study their swimming behaviour under a microscope. Afterwards, we will observe what happens when passive beads are added to the algal suspension, analyse those observations and adapt the experimental protocol (e.g. by varying the concentration and size of beads, the concentration of algae etc.) to prevent beads agglomeration or direct them at a targeted location.

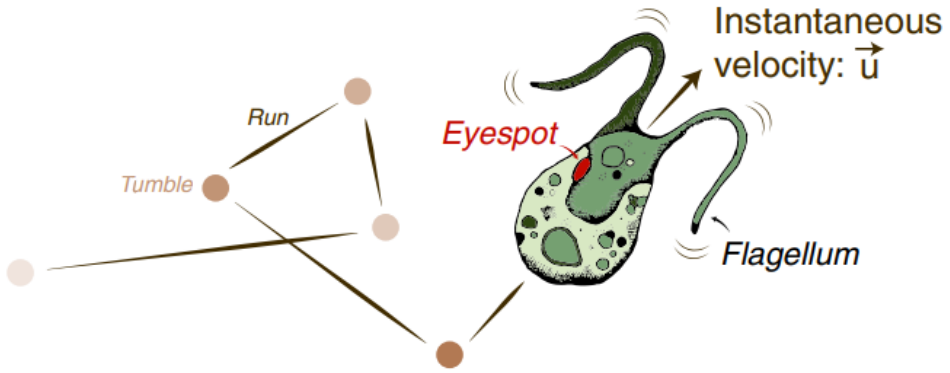

Figure 1.3: Sketch of a *Chlamydomonas reinhardtii*. From Ramamonjy *et al.* [5].

Chapter 2

Physics of microswimmers—single particle motion to phase separation induced

2.1 Flow generated by the swimming of a micro-organism

The research focuses on the study of microorganisms' swimming behavior in fluid environments similar to water. Given their micrometric sizes and speeds, which are approximately tens of microns per second, these microorganisms' swimming corresponds to low Reynolds numbers. In such scenarios, the Navier-Stokes equation simplifies to the Stokes equation[6].

The velocity field generated by a microorganism's swimming can be calculated as a superposition of singularities in the far field due to the linearity of the Stokes equation. For particles propelled by an external force, the singularity is termed a stokeslet, representing the Stokes flow generated by a point force.

However, for swimming microorganisms, the stokeslet is not an accurate representation as their net force is zero, with thrust and drag forces balancing each other. Therefore, the Stokes dipole, which consists of two closely placed stokeslets in opposite directions, is used to model micro-swimmers.

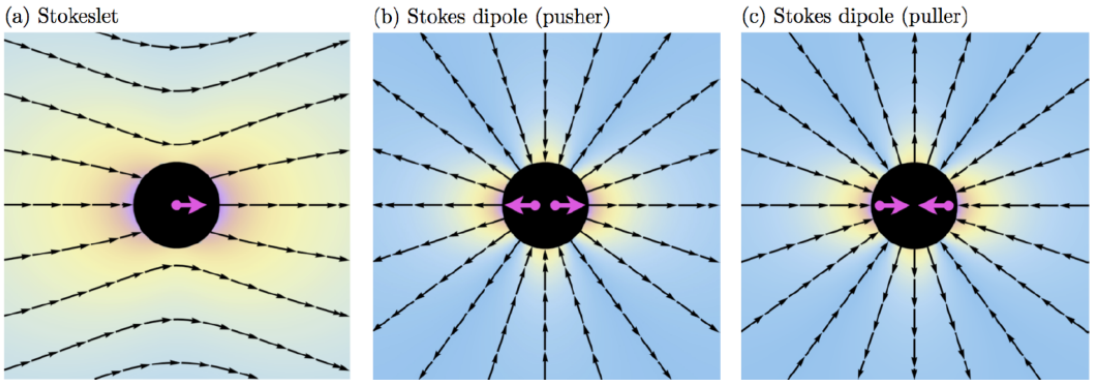

Figure 2.1: Schematic representation of mean velocity fields: a stokeslet (a), a dipole corresponding to a pusher (b) and a puller (c). From Bechinger *et al.* [3].

The research also differentiates between 'pullers' and 'pushers'[7]. For instance, the algae *C. reinhardtii*, which have two flagella and swim in a manner similar to breaststroke, are categorized as pullers. In contrast, bacteria like

Escherichia coli or soil bacteria *S. meliloti* and *B. contaminans*, which use flagella forming a propeller for forward movement, are classified as pushers[8].

We also notice the work of Drescher et al.[9], who tackled the challenge of measuring the average velocity field around several microorganisms using passive tracers. They measured the flow around an isolated *C. reinhardtii algae*, of size approximately 5 µm. This flow, visible in figure:2.2 on the left, is more complex than that of a simple puller. Due to the position of the flagella, which are relatively far from the body, the flow is more akin to a combination of three stokeslets, as can be seen in figure:2.2. The first one, directed to the right, corresponds to the body of the algae, while the other two, directed to the left, correspond to the two flagella.

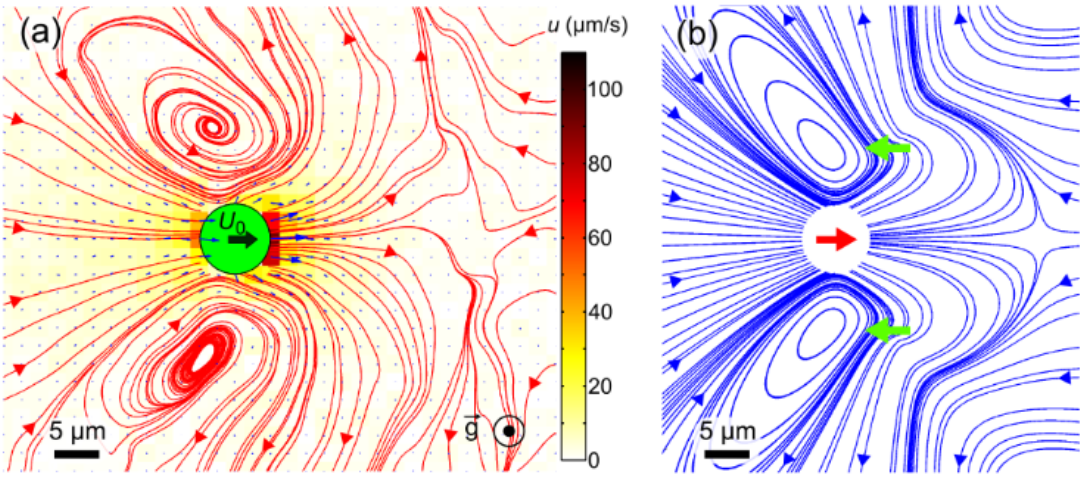

Figure 2.2: Average velocity field of the fluid surrounding an isolated micro-organism. (a) Around C. reinhardtii algae. (b) Modelling around three stokeslets represented by the red and green arrows. From Drescher *et al.* [9].

2.2 Motility induced phase separation

Dolai et al. numerically investigated the phase separation induced by motility (MIPS) in a mixture of active and passive particles interacting through weak repulsive forces[10].

They observe several regimes depending on the surface fractions of the two types of particles and the ratio between their sizes. These regimes include a homogeneous regime where the particles are uniformly distributed, an aggregation regime where clusters appear, and a phase separation regime where the passive and active particles are completely separated into two distinct phases. The study also finds an attractive force between the passive particles, induced by the motility of the active particles, similar to the findings of Angelani et al. They also examine the size distributions of the clusters in the different regimes.

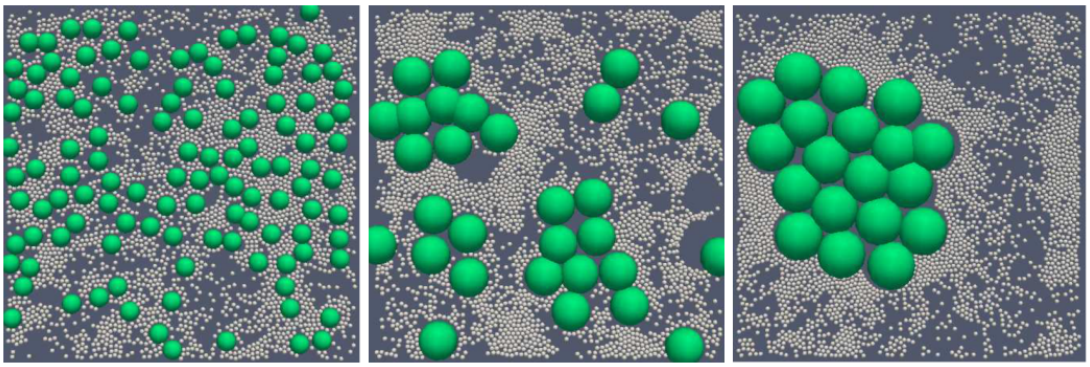

Figure 2.3: Images from numerical simulations of a mixture of active and passive particles. The surface fraction of passive particles is 0.3, that of active particles is 0.4 and their normalized activity is 0.125. The aspect ratio between passive and active particles is 4 (left), 8 (center) and 10 (right). From Dolai *et al.* [10].

Chapter 3

Experimental devices and methods

3.1 Preparation of cellular cultures

This passage describes the cell culture protocol used for cultivating the algae *C. reinhardtii*, which was developed by a previous intern, Hassan Madkour[11].

In its natural environment, *C. reinhardtii* follows a cycle of swimming during the day while performing photosynthesis and entering mitosis at night, during which it remains immobile. This cycle is replicated in a laboratory setting using an incubator with controlled luminosity and temperature, following a 12-hour day/night cycle.

Both solid and liquid cultures of *C. reinhardtii* are produced. Solid cultures are immobile aggregates of algae spread on agar gel containing TAP (Tris-Acetate-Phosphate) growth medium in a petri dish. These cultures are maintained weekly to ensure a constant supply of algae and to have a backup in case of contamination.

Liquid cultures are derived from solid cultures by introducing a small volume of the solid culture into liquid TAP culture medium. The cells reach peak growth within three days, after which a solution of swimming *C. reinhardtii* is obtained. To achieve monodisperse cellular suspensions with similar sizes, a part of the suspension is taken after the first three days and diluted within more TAP, then allowed to develop for three more days.

Before an experiment, 1mL of the algal suspension is centrifuged to separate the algae that resist gravity better, which should remove most of the dead algae and other impurities. This suspension is centrifuged again, and the bottom part is taken to obtain a concentrated and active algal population.

3.2 Description of the experimental set-up

Our system, sketched in Fig. 3.1, consists in fluorescent polystyrene beads (PS-FluoGreen) of diameters DB ranging between 2 and 40 μm added to a suspension of *C. reinhardtii*. A drop of the bead-bacteria mixture is injected in a chamber placed on the stage of an inverted microscope. The chambers were made by bonding a 25 μL FrameSealed Chamber (Bio-Rad), height 310 μm and side length 9 mm, on a glass slide covered by a silicon sheet (Gel-Film). This substrate limits the beads adhesion. The chamber is closed by a PDMS (polydimethylsiloxane) cover to ensure a good oxygenation of the suspension, and placed on the stage of an inverted microscope equipped with a (2048 x 2048) $pixels^2$ camera[1].

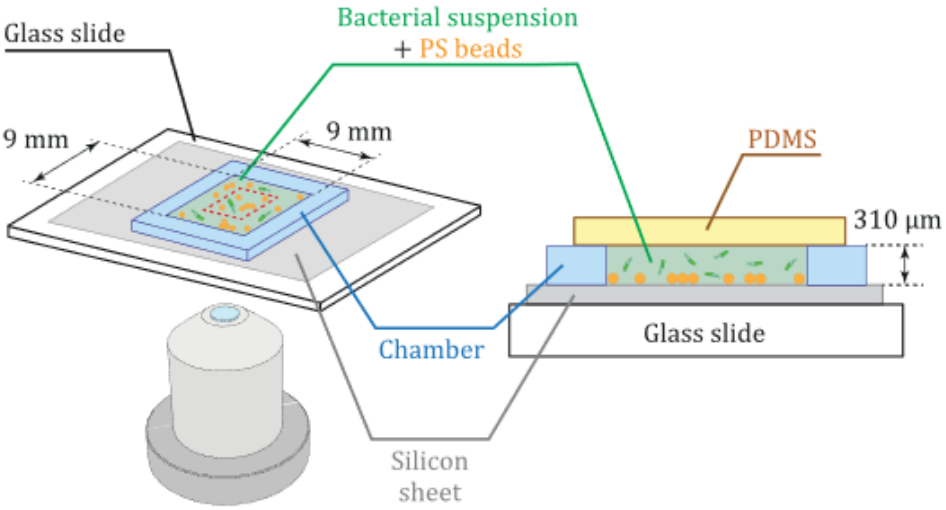

Figure 3.1: Experimental setup. From Bouvard *et al.* [1].

3.3 Trajectory reconstruction

To carry out a quantitative analysis of motility, we will utilize microscope films captured at various magnifications. In these films, we will identify visible bacteria in each frame and subsequently reconstruct their trajectories over time.

The recording of these videos is performed using either HCImage Live or LAS X (Leica Application Suite X) software, contingent on the microscope in use (we have two Leica DMI 6000 microscopes at our disposal).

Following the recording, the images are processed using Fiji (an open-source platform for biological-image analysis), where the Trackmate plugin[12] is employed to facilitate the detection of bacteria and the reconstruction of their trajectories. The resulting data are then exported to MATLAB for further analysis. Here, we have the flexibility to filter, sort, and visualize the data as needed.

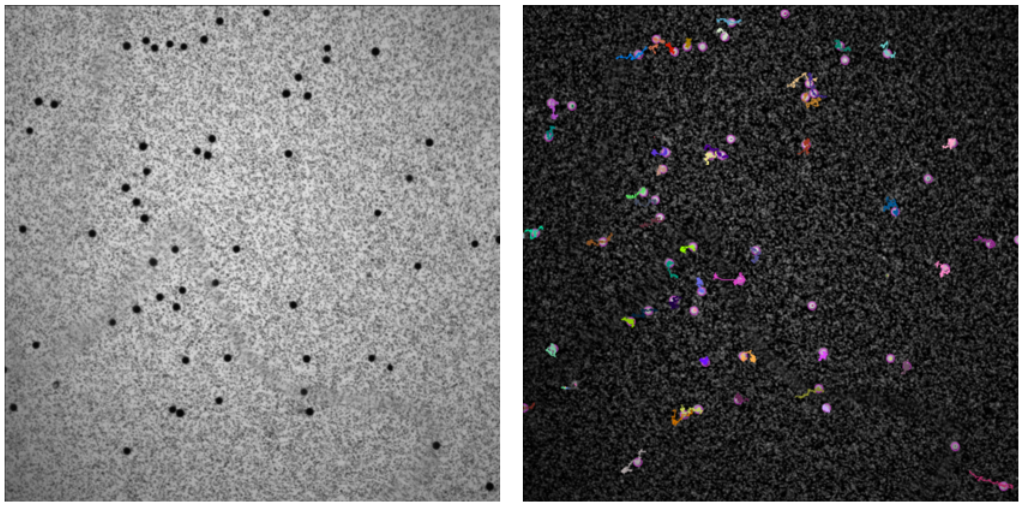

Figure 3.2: The processing performed on Trackmate is superimposed on the raw image: each purple circle corresponds to a detected bacterium, and the lines represent their trajectory over the last second.

Chapter 4

Diffusion coefficient

The Mean Squared Displacement (MSD) method is a standard technique used in the field of microbiology and biophysics to quantify the diffusive behavior or the random motion of particles such as bacteria, cells, or molecules within a medium. This is often done in the context of studying Brownian motion, chemotaxis, or other forms of movement.

The diffusion coefficient is a parameter that characterizes the speed of diffusion: the higher the coefficient, the faster the movement of particles. This parameter is essential in understanding the behavior of bacteria in different environments, such as how quickly they can spread in a host organism, how they navigate towards nutrients, or move away from harmful substances.

The formula to calculate MSD is:

$$MSD(\Delta t) = \langle |r(t+\Delta t) - r(t)|^2 \rangle \tag{4.1}$$

Where:

- $r(t)$ is the position of the particle at time t,
- Δt is the time interval,
- $\langle ... \rangle$ indicates the average over all time points.

The relationship between MSD and time can indicate the type of diffusion:

- If $MSD \propto \Delta t$, this indicates normal diffusion, often modeled as Brownian motion. The slope of MSD versus time gives the diffusion coefficient (D) in this case.
- If $MSD \propto (\Delta t)^2$, this indicates ballistic or directed motion, not diffusive.
- If $MSD \propto (\Delta t)^\alpha$ with $\alpha \neq 1$, this indicates anomalous diffusion. When $\alpha < 1$ it's subdiffusive and when $\alpha > 1$ it's superdiffusive.

In the study conducted by Xiao-Lun Wu and Albert Libchaber[13], the influence of bacterial motion on micron-scale beads in a freely suspended soap film was investigated. Given the sizes of the bacteria and beads, the experimental geometry was effectively two-dimensional.

Significant positional fluctuations were observed for beads with diameters as large as 10 µm. The measured mean-square displacements (MSDs) suggested an interesting diffusive behavior: superdiffusion in short time scales and normal diffusion in long time scales. Although this phenomenon bears some resemblance to the Brownian motion of small particles, its underlying physical mechanisms are distinct and are ascribed to the collective dynamics of bacteria.

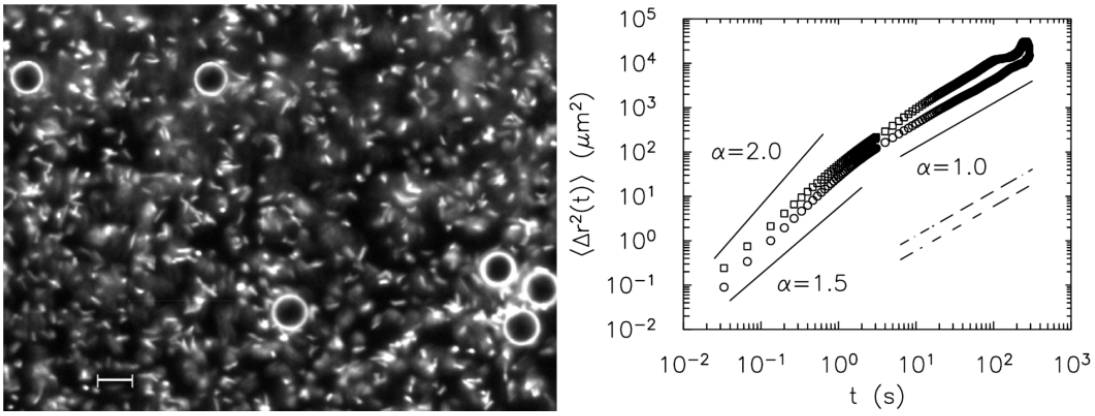

Figure 4.1: Fluorescence image of 10 µm polystyrene beads in a suspension of suspension of fluorescent *E. coli* bacteria and Mean square displacement (MSD) of polystyrene beads with diameters of 4.5 µm (squares) and 10 µm (circles). From Wu and Libchaber *et al.* [13].

Therefore, I conducted experiments using different concentrations of *C. reinhardtii* and beads(figure:4.2).

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Figure 4.2: List of experiments.

It can be found that some of the results are not such as Wu et al[13]. By analyzing the film, we found that what we want to characterise is the motion of beads induced by the swimming of algae. In order to do that, we need to be in a dilute regime of beads to avoid bead-bead interactions. In my movies, the concentration of beads is too high and there are lots of interactions between beads(figure:4.3). So the MSDs we have plotted, show not only the motion of the beads induced by the swimming of algae, but also the confinement of the beads(figure:4.4).

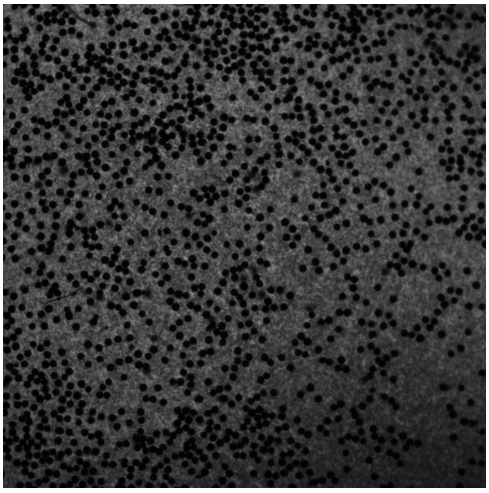

Figure 4.3: There are too many beads in the chamber, which causes beads to collide with each other.

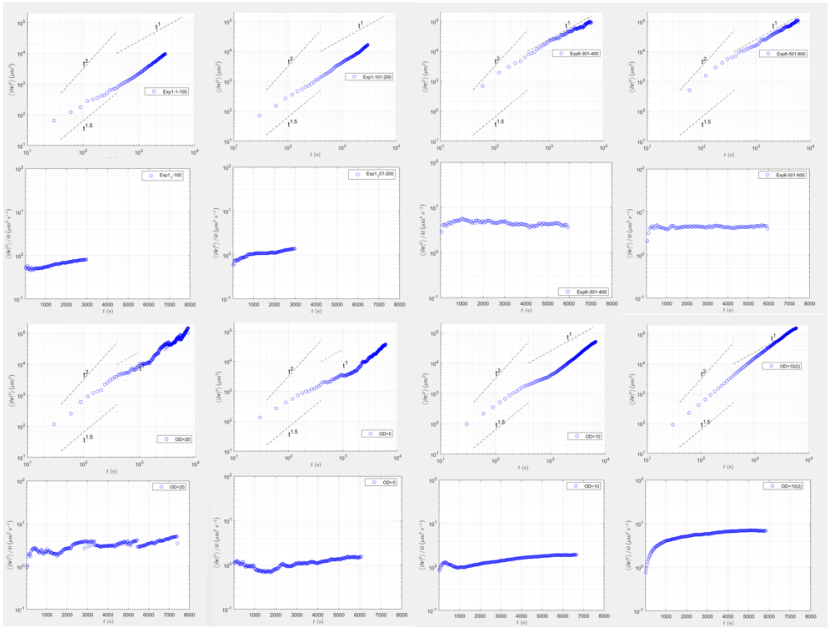

Figure 4.4: Some of the results of MSD.

As can be seen from the curves of figure:4.5, the MSD reveals the existence of two regimes. The first, at short time $t < 10^2 s$, is characterized by a power law in t^δ with δ between 1.5 and 2. They named this regime 'super-diffusive', in contrast to the second, long-time regime ($t > 100s$), which exhibits a power law typical of a diffusive system with $\delta = 1$. The D_{eff} ($= \mu_B$) of polystyrene beads derived from here also agrees with the results of Kyriacos C. Leptos et al.(figure:4.6)[14].

Afterwards we calculated D_{eff} using this formula 4.2 and plotted it in a graph with the OD variation and it can be seen that the results were compared with Leptos[14] and both were found to show a linear correlation with the concentration of *Chlamydomonas reinhardtii*.

$$\mathrm{MSD}(t) = \left\langle \left\langle \|\vec{r}(t_0 + t) - \vec{r}(t_0)\|^2 \right\rangle_{t_0} \right\rangle_{\text{bead}} = 2d\mu_{msd}t \tag{4.2}$$

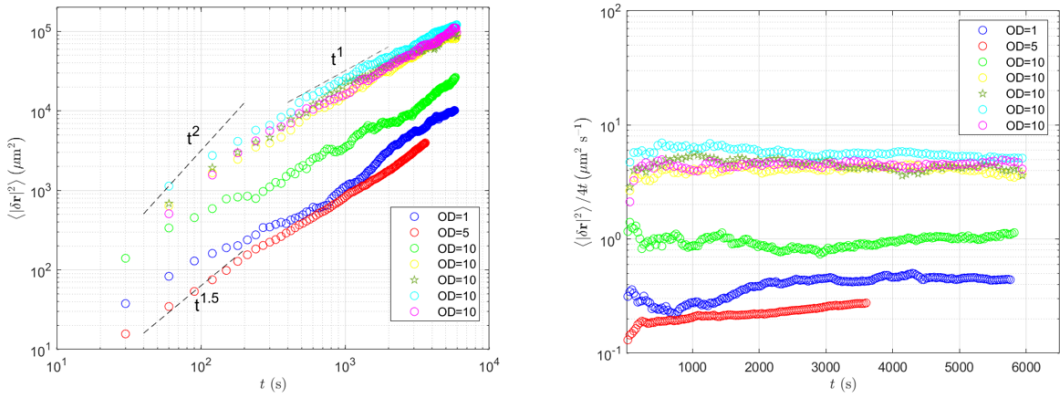

Figure 4.5: Good results of MSD.

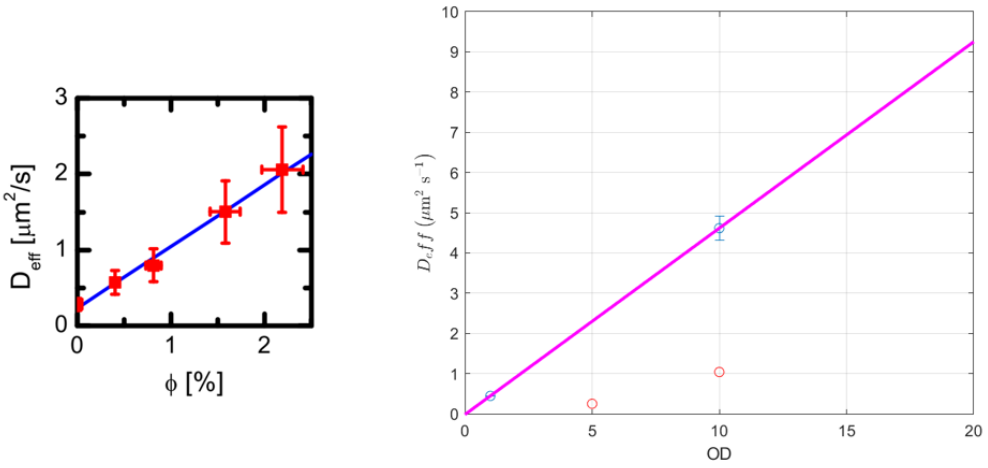

Figure 4.6: Concentration dependence of (b) effective diffusivity, with linear fit, and (c) fractional contribution of PDF tails. From Leptos *et al.* [14].

OD	D_{eff} (μm^2 s^{-1})
1	0.445136
5	0.249385
10	1.038811
10	4.08591
10	4.344138
10	4.536224
10	5.498108

Table 4.1: Table of OD and D_{eff} values

Chapter 5

Conclusion

This study provides significant insights into the intricate dynamics and diffusive behaviors of microorganisms, with a particular focus on the unicellular algae *C. reinhardtii*. By meticulously analyzing the movement patterns and interplay of these organisms in various environments, the research enhances our understanding of their navigation capabilities and survival strategies.

The study highlights two distinct diffusive regimes: a short-time super-diffusion regime characterized by a power law with exponents ranging between 1.5 and 2, and a long-time normal diffusion regime characterized by an exponent of 1, indicative of standard diffusive behavior. The results not only validate prior research but also introduce fascinating new observations, such as the occurrence of a third regime in certain instances, which warrants further investigation.

However, the research is not without its challenges and limitations. The high concentration of beads used in the experiments occasionally led to unwanted interactions, causing a deviation in the Mean Squared Displacement (MSD) results. Hence, future studies should focus on adjusting the bead concentration to avoid such complications and enhance the accuracy of the results. Furthermore, the exploration of model building and the reproduction of the *Burkholderia contaminans* clustering phenomenon using *C. reinhardtii* offer promising avenues for future research.

This research journey, more than just an academic pursuit, has been a pathway towards professional growth and learning. It has honed my experimental acumen, introduced me to the captivating field of microfluidics, acquainted me with the works of acclaimed scientists like Eric Lauga, and granted me a practical understanding of the multifaceted applications of biofluidics.

In conclusion, as we delve deeper into this field, it's plausible to anticipate significant advancements in domains ranging from microbiology to biomedical engineering, demonstrating the far-reaching potential of these studies.

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