

**NOVEL PROPERTIES AND EMERGENT
COLLECTIVE PHENOMENA OF ACTIVE FLUIDS**

A DISSERTATION

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Acknowledgements

Dedication

To my beloved family for supporting me over the years.

Abstract

An active fluid denotes a suspension of particles, cells and macromolecules that are capable of transducing free energy into systematic motions. Converting energy at individual constituent scales, these systems are constantly driven out of equilibrium and display unusual phenomena, including a transition to a zero viscosity superfluid-like state and a transition to a collective moving turbulent state. These curious transitions are direct consequences of the motions of active particles, and are absent from classical complex fluids without self-propulsion. By elucidating the causes and consequences these phenomena, we will not only expand the knowledge of complex fluids, but also provide deeper understandings on the biological and ecological impact of living organism behavior.

In this thesis, experimental investigations on the rheology of active fluids is presented. Specifically, the viscosity of bacterial suspensions is significantly reduced by confining walls. We show that this effect results from upstream swimming bacteria near the confining walls, which collectively exert stress on the fluids.

The collective motions in dense bacterial suspensions are investigated. In particular, we present the first experimental study on the giant number fluctuation - a landmark of collectively moving active particles - in 3-dimensional bacterial suspensions. Our measurement agrees in part with theoretical predictions: measurement is consistent with theory at low and high concentrations, but displays a curious deviation from the theories at intermediate concentrations. We show that the deviation results from a strong interplay between the flow induced by bacterial motions and giant number fluctuations, which is absent in existing theories.

In addition, we measure the critical conditions of the transition from disordered state

to turbulent state in bacterial suspensions. We present the experimental results in a phase diagram, serving as a benchmark for existing and future theories. We put forward a heuristic model based on two-body hydrodynamic interactions, hoping to understand the transition in a more intuitive way and to stimulate theoretical advancement.

Our experiments allow quantitative understanding of active fluids and lay the foundation of applying active fluids to real world challenges.

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Chapter 1

Introduction

- Chapter 1 briefly describe the history and significance of active fluid research.
- Chapter 2 presents the experimental techniques used in this theis.
- Chapter 3 talks about one of the emergent properties: reduced viscosity. Large portion of this chapter have been published in [1].
- Chapter 4 talks about another emergent property: giant number fluctuation. This work is under preparation for submission.
- Chapter 5 presents the study on the transition from disordered state to active turbulence in light-powered bacterial suspensions. This work is conducted with a close collaboration with Yi Peng and Xiang Cheng. Large portions of this chapter has been published in [2]. Yi Peng, Zhengyang Liu and Xiang Cheng conceived the experiment. Zhengyang Liu constructed the light-powered bacteria. Yi Peng performed the experiment. Zhengyang Liu and Yi Peng did the data analysis. All authors contribute to the model development and writing of the manuscript.
- Chapter 6 summarizes the contributions of this thesis and provides the outlook

on future research.

- Appendix A shows details of the construction of light-powered *E. coli*.
- Appendix B provides details of several particle tracking tools I developed.
- Appendix C shows details of photolithography.

1.1 Active Matter and Active Fluids

Active matter denotes a large group of active units which utilize ambient energy to achieve motions. Examples include flocking birds, schooling fish, herding beasts and even human crowds, down to actin filaments powered by motor proteins, bacteria and chemical reaction driven particles [3, 4, 5, 6, 7, 8, 9]. The concept roots from a broader class of matter: soft matter, which includes polymers, surfactants and colloidal grains and shares common properties such as complexity and flexibility [10]. Like soft matter, active matter is also complex and flexible. What makes them more complex is the self-propulsion of each individual constituent, which endows them with more intriguing and counter-intuitive properties, challenging our understandings [11].

Active fluids, sometimes referred to as active gels, are suspensions of active agents such as cells, particles and biological macromolecules that are capable of utilizing chemical energy to sustain their self-propulsion. They are a subset of active matter, and the "fluids" in the name suggests the important role of the viscous hydrodynamic interaction and stress, in contrast to dry active matter [6]. The first glimmering of active fluids dates back to 1969, when Finlayson and Scriven found that motion could spontaneously set in a previously still material without the intervention of outside forces, due to composition-dependent stress [12]. However, the study on active fluids did not bloom, until 26 years later, when the seminal paper on modeling collective flocks came

out [13]. From then on, physicists are getting unprecedentedly interested in biological phenomena, leading to the emergence of a new field of study - active fluids.

Early accomplishments in the research of active fluids include two successful theoretical predictions on the abnormal rheology and the spontaneous active turbulence [14, 15], which were then demonstrated in quite a few experiments [16, 17, 18, 19, 20, 21]. From these beginnings, the field has been enjoying a vibrant interplay between experiment and theory, and more complex environment and geometrical constraints have been investigated [22]. As of now, the study of active fluids has provided us with a good qualitative understanding of some biological processes, such as how active turbulence enhances nutrient transport.

There are two promising directions in active fluids. One is to get quantitative understanding of the novel properties. These works will not only provide more accurate predictions on new systems, but also guide the engineering of artificial robots that can perform tasks in complex environment, such as drug delivery. Another direction is to invite chemistry and biology to collaborate on this highly interdisciplinary subject. A complete understanding of the behavior and properties of living systems will require the knowledge of biochemical signaling, which opens the door of an ambitious mission: elucidating tissue dynamics and developmental biology [6, 23]. The works that are to be described in Sec. 3, 4 and 5 are along the first direction: seeking more quantitative understanding of rheology and active turbulence of active fluids.

1.2 Novel Properties

Active fluids exhibit novel properties such as reduced viscosity and enhanced diffusion [4]. The reduced viscosity is induced by the force exerted by the swimming mechanisms of the active agents, such as bacteria and algae [27]. And the enhanced diffusion is attributed to the interaction - steric collision or hydrodynamic perturbation - between

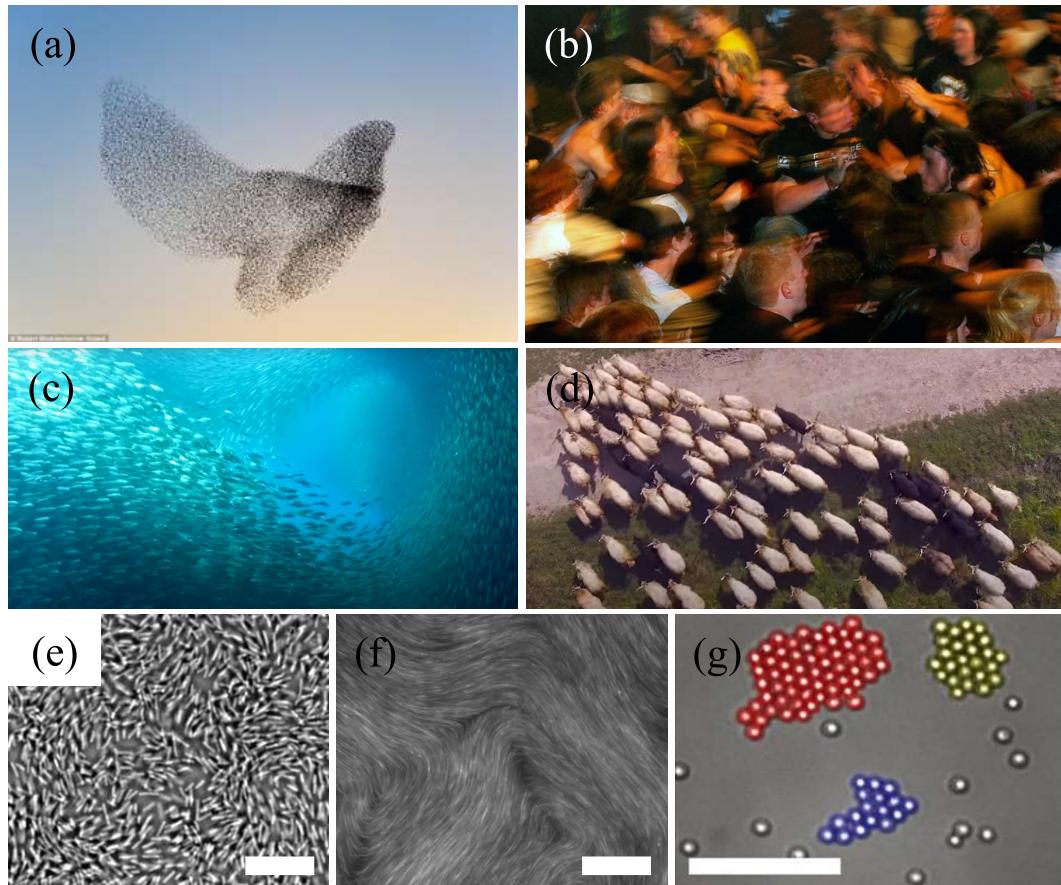


Figure 1.1: **Examples of living matters and active fluids.** (a) Flocking birds, (b) people in a mosh pit at heavy metal converts, (c) schooling fish, (d) herding sheeps, (e) swarming bacteria (f) microtubule and (g) clustering active Janus particles. Scalebars in (e) and (g) are $10 \mu\text{m}$. Scalebar in (f) is $200 \mu\text{m}$. Images courtesy of Robert Wolstenhome (a), Ulrike Biets (b) [24], biographic (c, d), DeCamp (f) [25] and Palacci (g) [26].

tracer particles and swimmers [28, 29, 30, 31, 32, 33, 34, 35, 36]. In this section, the existing works regarding rheology and diffusion in active fluids are reviewed, and motivations for investigating the rheology of bacterial suspensions under confinement (Chap. 3) will be discussed.

1.2.1 Rheology

Viscosity of a fluid can be understood as its resistance to flow. When flowing, fluid elements move relative to others, resulting in energy dissipation due to friction. The more energy is required, the more "viscous" the fluid is known to be. A suspension of passive particles is always more viscous than its suspending fluid, a fact that was first formulated by Einstein in 1906 [37]. Recently, the study of active fluids revealed that active particles modify the viscosity of their suspending fluids in a different and interesting way.

In 2004, Hatwalne et al. predicted that micro-swimmers, depending on their self-propelling mechanisms, can modify the suspension viscosity in different ways [14]. Most common micro-swimmers, such as unicellular microorganisms, can be classified into two types: pushers and pullers, based on the far field flow they generate. Fig. 1.2b illustrates the most common pushers and pullers in nature: bacterium and algae. If one puts a elongated rod-like bacterium in a simple shear flow, as illustrated in Fig. 1.2a, the preferred orientation of the bacterium is along the extensile flow [38]. Such an orientation makes the flow generate by the swimming bacterium coincide with the imposed shear flow, and thus compensating the viscous dissipation of energy, which effectively reduces the viscosity. In contrast, in the case of puller swimmers, such an orientation makes opposites the directions of swimming induced flow and imposed shear flow, which enhances the viscosity.

Their prediction was confirmed by numerical solutions of the theory [39, 40] and

experiments [19, 20, 21]. Cates et al. reached at the same conclusions as Hatwalne et al. did: while contractile gels exhibit a divergence of apparent viscosity, extensile gels show a zero-viscosity phase. Giomi et al., on top of these results, emphasized the important role of particle shape. They showed, in their numerical study, an rheological equivalence between rod-like pusher swimmers and disk-like puller swimmers (see Fig. 1.2c). In particular, they predicted a thickening effect of spherical puller swimmers (corresponds to the 0 shape parameter in Fig. 1.2c). This prediction was later on challenged by another theory in the framework of swim stress, which predicts a viscosity reduction of a spherical pusher swimmer suspension [41]. Due to the difficulty of synthesizing large amount of artificial swimmers, this debate has not been resolved yet. However, with the rapid development of synthesizing techniques [26, 42], it is getting more promising that we will resolve it, and formulate a more complete understanding on how active swimmers modify the rheology.

Experimental confirmation of these predictions posed challenges on traditional rheometries due to the tiny shear stress that is required to be measured. As a result, new rheometries are needed [43]. In 2009, Sokolov and Aranson came up with an innovative way of measuring the such tiny stress [19]. By moving a probe in a suspension of *Bacillus subtilis* bacteria, a pusher type swimmer, they generated a large vortex. By studying the decay of the vortex, they got a measure of the viscosity. For the first time, they experimentally confirmed that pusher swimmers reduced the viscosity (see their results in Fig. 1.2d). In 2013, Gachelin et al. adopted a microfluidic viscometer to measure the viscosity of suspensions of *Escherichia coli* bacteria, another pusher type swimmer [20]. They confirmed again the viscosity reduction. A more remarkable finding is the non-newtonian behavior: the viscosity was reduced at low shear rate, but was enhanced at high shear rate. This observation suggested that it is the competition between bacterium intrinsic shear rate and the impose flow shear rate that determines

how the viscosity is modified. In 2015, Lopez et al. published arguably the most important experimental work on the rheology of active fluids, which showed that the apparent viscosity of an *E. coli* suspension can be reduced to zero if the swimming activity is sufficiently high [21]. The authors modified an old-fashioned Couette concentric cylinders, where the outer cylinder was set to rotate at a fixed rate and the torque on the inner cylinder was measured. The high sensitivity was achieved by using a string that was highly sensitive to torque to hang the inner cylinder, so that a stress, as small as that generated by bacterial suspensions, can be detected (see their rheometer and results in Fig. 1.2e-f). The authors termed their zero-viscosity suspensions “superfluids”.

Despite the great progress on the rheology of active fluids made so far, there remains complexity that is not readily understood. Confinement, or more generally boundary conditions or geometry, is one of the leading factors that contributes to this complexity. The behavior of active particles can be altered greatly by confinement. In 2005, Voituriez et al. showed theoretically that a spontaneous flow transition from a homogeneous immobile state could happen in active polar gel under confinement [44]. Such spontaneous flow transition was confirmed in both numerical and experimental studies [45, 46, 47]. The complexity introduced by geometry was also manifested by the experiments where single bacterial vortex was stabilized by confinement [48, 49, 50] and where asymmetric gears were powered by swimming bacteria [51, 52]. The effect of confinement on the rheology of active fluids was first studied theoretically based a kinetic theory [53] and a generalized Navier-Stokes model [54]. In this thesis, I will present the first experimental study on active fluid rheology using bacterial suspensions (Chap. 3). The fact that our experimental results agreed with neither theory manifested the complexity and the lack of understanding of active fluids. Together with the experimental results, we provided a heuristic model that qualitatively captured the rheological properties and hope to stimulate further theoretical studies on this matter.

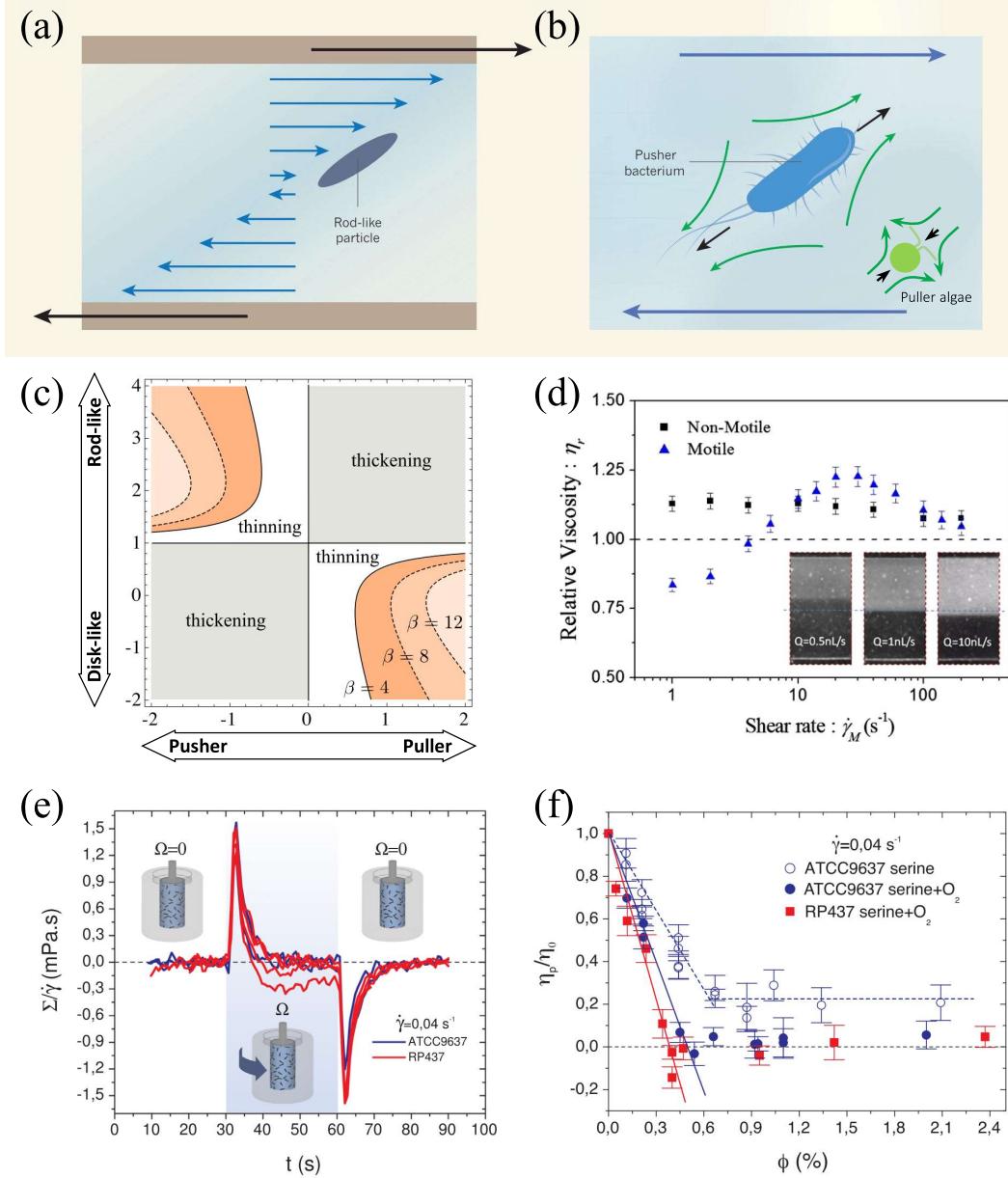


Figure 1.2: Rheology of active fluids. (a) The preferred orientation of the bacterium is along the extensile flow. (b) The most common pushers and pullers in nature: bacterium and algae, and their corresponding far field flow. (c) Rheological effect phase diagram of swimmer shape and swimming mechanism. (d) Non-Newtonian behavior of *E. coli* suspensions. (e) Stress response of *E. coli* suspensions in a modified Couette concentric cylinder rheometer. (f) Viscosity of *E. coli* suspensions at various volume fractions. Image courtesy of Marchetti (a, b) [43], Giomi (c) [40], Gachelin (d) [20] and Lopez (e, f) [21].

1.2.2 Diffusion

The diffusion of passive particles in active fluids, such as nutrients and signaling molecules, are significantly enhanced. Such enhancement has been shown to have great biological and ecological importance [28, 36, 31], as well as to provide a useful tool of probing novel properties of active fluids [55]. Unlike the study of rheology, which was initiated by theoretical prediction, the study of enhanced diffusion started from an experiment.

In 2000, Wu and Libchaber studied the diffusion of spherical polystyrene particles in a bath of actively swimming *E. coli* [28] (see Fig. 1.3a for their experimental system). They characterized the diffusion of the tracer particles by measuring their mean squared displacement (MSD) (see Fig. 1.3b for typical MSD data), and reported two findings: 1) the MSD exhibits a superdiffusive regime at short time, which is followed by a diffusive regime at longer time; 2) the effective temperature, backed up from effective diffusion coefficient using Stokes-Einstein equation, is several order of magnitude larger than room temperature. Their qualitative findings were confirmed by computational [56, 57], theoretical [58] and other experimental studies [59, 33, 60, 36, 32]. A remarkable progress towards quantitative understanding was made by Mino et al., who experimentally identified that the enhancement of diffusivity is proportional to the "activity flux" (defined as concentration multiplied by the mean velocity of swimmers). This model was further developed to capture the experimental result more accurately and to account for more complex conditions [61, 62, 31].

Although a lot of progress has been made on understanding diffusion isotropic particles (spheres) in an active bath, how anisotropic particles diffuse remained largely unexplored. Yet, the diffusion of anisotropic particles has both fundamental and application significance. On the one hand, it was shown that the Brownian motion (i.e. in a passive bath) of anisotropic particles exhibited a subtle interplay between orientational and translational motions [63]. Previous studies preferred to consider an active bath

equivalent to a high temperature passive bath [28], and it was shown to be a good equivalence for isotropic particles. A fundamentally interesting question to ask is: does active bath also alters the interplay between orientational and translational motions? The answer is yes, and we will see that this interplay is specific to swimming mechanisms. On the other hand, few particles or molecules in nature are perfectly isotropic. Generalizing the enhanced diffusion to anisotropic particles will have significant impact on applying this knowledge to real world problems. In 2016, Peng et al. studied the diffusion of polystyrene ellipsoids in a quasi-2D free-standing soap film of *E. coli* bath (see setup schematic in Fig. 1.3c) [29]. In contrast to the pure Brownian motion, where ellipsoids preferred to diffuse along their major axes [63], they found that an active bath forced the ellipsoids to move primarily along the minor axes (see Fig. 1.3d-e for a comparison). This phenomenon was explained by considering the far-field dipole flow of a single *E. coli* bacterium. An interesting prediction naturally arises: in a bath of puller swimmers, whose far-field flow is opposite to that of pushers, the diffusion of ellisoidal tracers should be primarily along the major axes. This prediction was later on proved experimentally by Yang et al. [34] in a green algae *C. reinhardtii* bath and I was involved in this work. Due to the fact that my contribution was relatively small to this work, I will not provide more details about it in my thesis. However, readers interested are encouraged to find out more in our original paper [34].

1.3 Collective Motion and Giant Number Fluctuations

Collective motion is defined as an emergent directed movement in a large number of animals or particles, which are capable of moving on their own. In the seminal paper by Reynolds published in 1987, he tried to use a simulation approach - rather than scripting the paths beforehand - to generate vivid motions of animals in computer graphics [64]. This idea has soon evoked enormous research interest of physicists because a seemingly

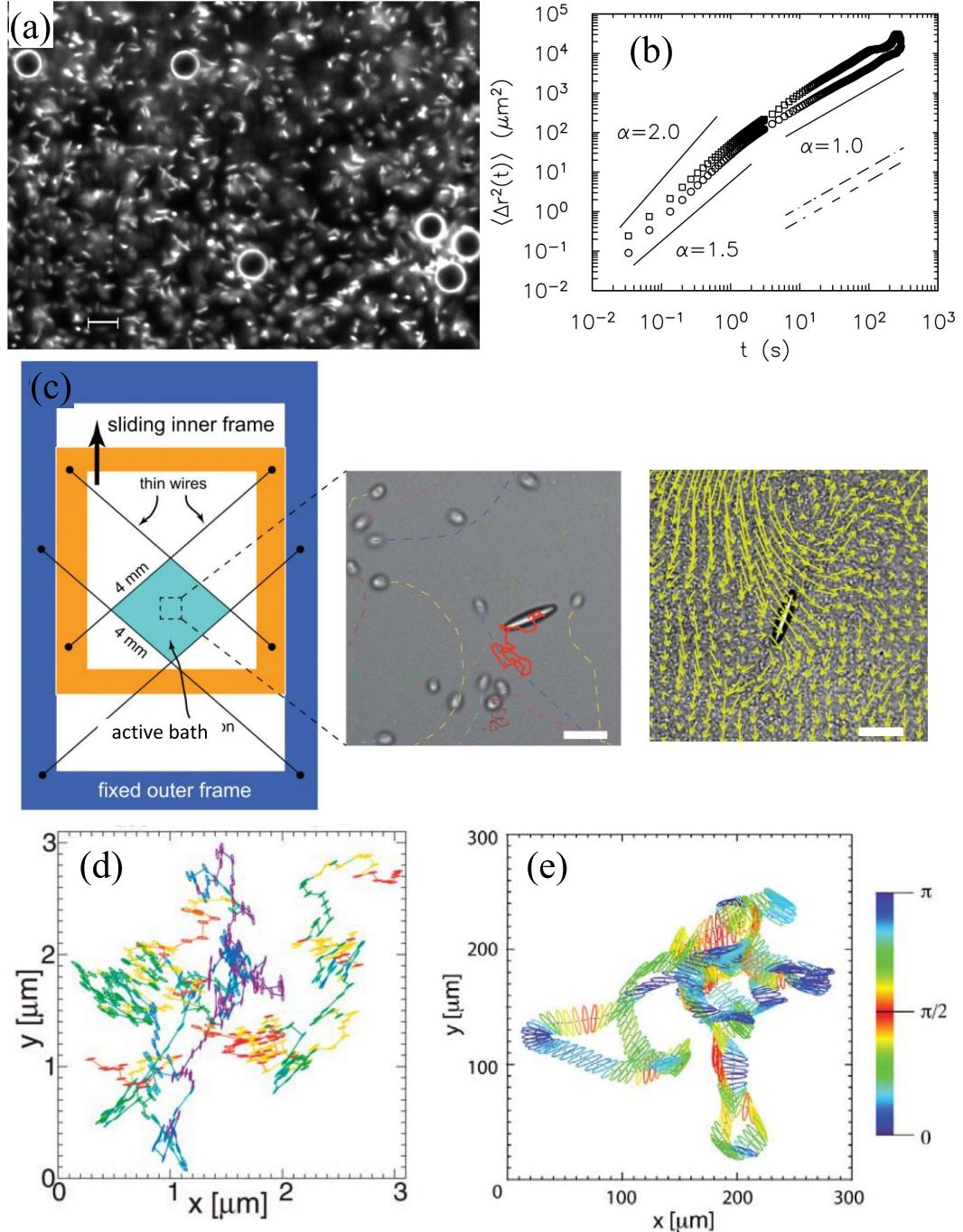


Figure 1.3: Diffusion of passive tracers in an active bath. (a) 10 μm diameter PS particles suspended in a bath of *E. coli* (b) Mean squared displacement (MSD) of tracer particles as a function of lag time. (c) Free-standing film setup adopted by Peng et al. and Yang et al. (left)[29, 34]. A microscopic image of ellipsoidal PS particle suspending in *C. reinhardtii* (middle) and *E. coli* (right) baths. Scale bar: 20 μm . (d) and (e) present both the translational and orientational trajectories of ellipsoids diffusion in water and *E. coli* bath, respectively. Image courtesy Wu (a, b) [28], Yang (c) [34], Han (d) [63] and Peng (e) [29].

universal animal behavior was found in animals across different length scales: large animals as birds and fish, all the way down to insects or even microorganisms.

In this section, we first review recent progresses on understanding the collective motions in various living and non-living systems. Our research on "imaging the emergence of active turbulence" will be motivated (detailed in Chap. 5). Then, I will describe an important consequence of the collective phenomena: giant number fluctuations, and I will motivate our research on "giant number fluctuations in 3-dimensional bacterial suspensions" (detailed in Chap. 4).

1.3.1 Collective Motion

The research on collective motion dates back to the 1980s, when flocking birds, schooling fish, herding beasts and even human crowds (Fig. 1.1a-d) were regarded as an orientationally ordered phase of living matter, in analogy with ferromagnetic spins [64, 13, 65, 66, 67, 24]. Besides macroscopic systems mentioned above, smaller and more laboratory accessible model systems have joined this family and have been studied extensively. As examples, actin filaments and bacteria exhibit turbulence-like swirling patterns, and synthetic active colloids form dynamic clusters (Fig. 1.1e-g) [68, 17, 69, 26, 70, 71, 72].

While being fascinated by the patterns formed by collectively moving animals or particles, researchers are trying to come up with rules, models and theories to explain and predict this process. There is a vast literature trying to understand collective motions in different systems from various approaches. To get an idea of how extensively it has been studied, Ref [64] by Reynolds has been cited more than 10,000 times so far. I will briefly review the attempts to model collective motion, which are most relevant to our research that will be detailed in the following chapters. A more comprehensive review on the studies of collective motions can be found in the review paper by Vicsek [5]. In 1987, Reynolds came up with arguably the first set of rules to capture the features of

collective motions, based on a simple self-propelled particle model. Three rules were set into the system: separation, alignment and cohesion, as illustrated in Fig. 1.4a-c, where the blue and green triangles in are the actively moving particles in his simulation, called “boids” [64]. In 1995, Vicsek modified Reynolds’ model by replacing the separation and cohesion rule with a random perturbation in the velocity of each particle, while keeping the alignment rule, which dictates one particle to always point to the same direction as its neighbors [13]. Due to the simpler and more robust rules compared to Reynolds’ model, Vicsek’s model was able to simulate huge flocks. In later studies, the Vicsek Model has become a standard model where properties of collective motions are explored [73, 74, 75, 76, 77]. Despite the success of discrete and finite system simulation in understanding 2D collective motions, it was challenging to study long range, long time and higher dimensional dynamics using such approach. In 1995, Toner and Tu wrote down a continuum equation of motion for a “large universality class of models” such as the Vicsek Model based on symmetry and conservation arguments [78]. In 2002, Simha and Ramaswamy made the first attempt to address hydrodynamic effect in self-propelled particle suspensions [15]. Within the framework of liquid crystal physics, they formulated an equation of motion with hydrodynamic terms to account for the “flow-alignment” effect [38]. The inclusion of hydrodynamic effects turned out to be very important, because it directly accounted for phenomena in bacterial suspensions, the largest group of prokaryotic organisms and the most studied model active system. Their theory captures several aspects of bacterial suspensions well, including the emergence of active turbulence and giant number fluctuations as a consequence of the bend instability of an ordered state (illustrated in Fig. 1.4d). In 2008, Saintillan and Shelly adapted kinetic theories, which were previously used to study polymers, to study the suspensions of self-propelled particles [79, 80]. Their theory generalized the predictions by Simha and Ramaswamy by predicting that, in addition to ordered state, a disordered state is

also unstable and can evolve into a turbulent state. Moreover, they made interesting investigations on the nonlinear effects, such as pattern formation and efficient fluid mixing. Fig. 1.4e shows one instance of their simulation on the evolution of fluid mixing driven by active pusher swimmers. There are other attempts to model the collective motion and its consequences that I haven't included. For a more comprehensive review of both theoretical and experimental advances, see the review papers [4, 81, 6].

As of today, these universal patterns can be qualitatively reproduced by simple models with collision rules and noise. And quantitative description is developing with more observations available, which is bound to have impactful applications, including understanding the reaction of panic crowd and predicting the migration of fish schools [5].

In my research, bacterial suspensions are the model system. In such system, the collective motion is usually manifested by the emergence of “active turbulence”, where vigorously swimming bacteria are stirring the fluid and generating vortex- and jet-like patterns, reminiscent of high Reynolds number turbulence [16, 72, 19, 82, 83, 17, 68, 2]. The onset of large scale collective motions in bacterial suspensions has been regarded as a disorder-order phase transition, where critical conditions at which the transition should happen have been predicted by various theoretical works [84, 81, 6, 85]. In contrast to the abundance of theoretical predictions, definitive experiments that can quantitatively verify them are still lacking [81, 85]. To fill this gap, I worked closely with my colleague Dr. Yi Peng on systematically measuring the critical conditions of this transition. The result will be presented in Chap. 5.

1.3.2 Giant Number Fluctuations

Active particles displays transient large scale inhomogeneity even in a *statistically homogeneous and stationary state*. Such a phenomenon is referred to as *giant number*

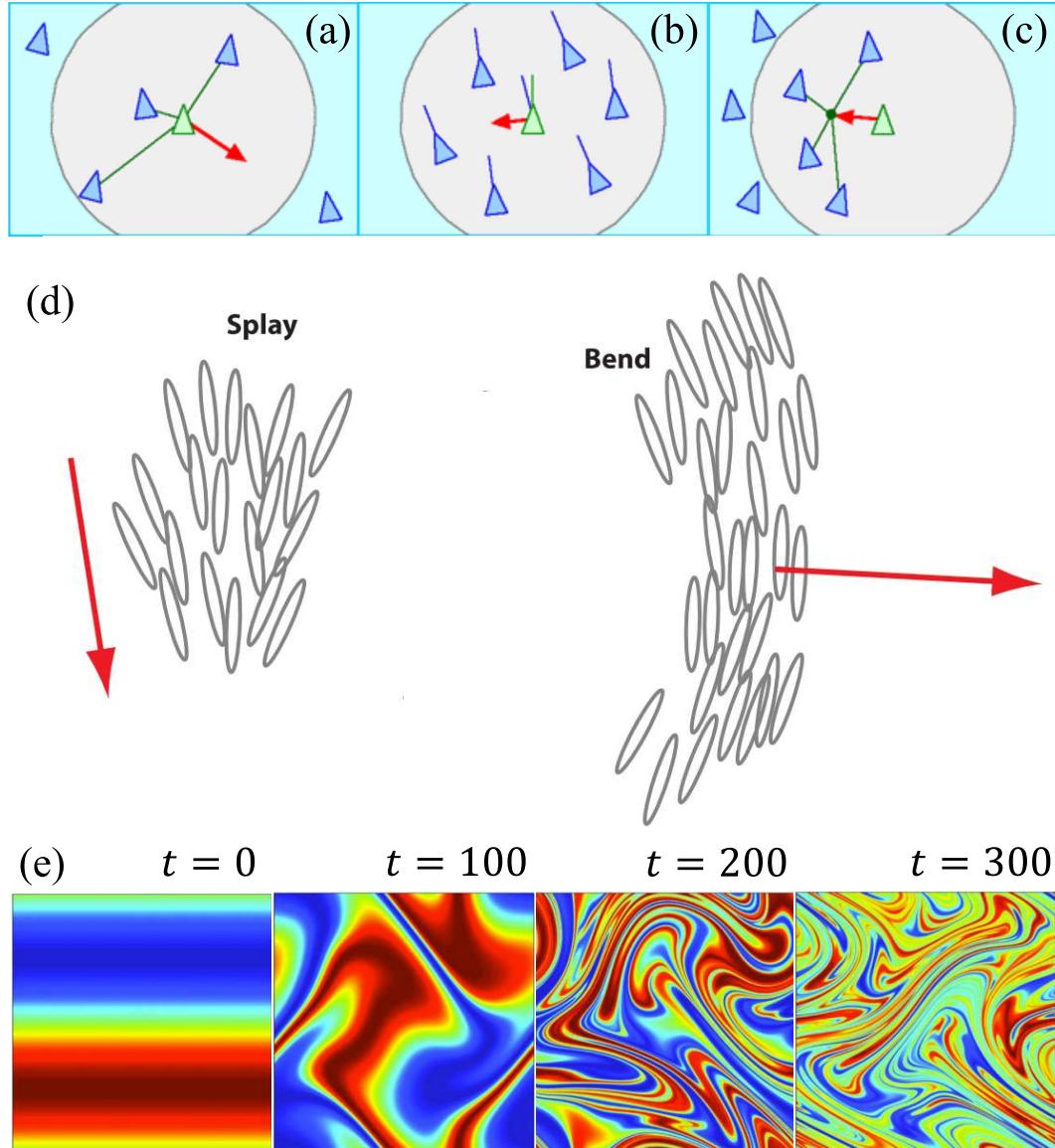


Figure 1.4: Illustrations of existing models and theories of collective motions. (a)-(c) Reynolds' “boids” model obeying the rules of separation (a), alignment (b) and cohesion (c). (d) Nematic self-propelled particle model, displaying splay (left) and bend (right) instability. (e) Simulation of efficient fluid mixing driven by active pusher swimmers. Image courtesy of Reynolds (a-c) [64], Ramaswamy (d) [4] and Saintillan (e) [80].

fluctuations (GNF). Formally, it is defined as the anomalously strong dependence of the variance of the number of particles on the mean number.

Chapter 2

Experimental Background

In this chapter, experimental techniques that are used in my research will be described briefly as a practical guide for those who want to test or perform some parts of the experiments in this thesis. The following aspects will be covered:

- *Escherichia coli* (*E. coli*) bacterial suspensions are the model throughout the whole thesis, so I will start talking about the preparation of motile bacterial sample in Sec. 2.1.
- Optical microscopy along with digital imaging has been the key approach for investigating the properties of bacterial suspensions. Such approach naturally demands automated image analysis tools. In Sec. 2.2, I will describe in detail some new techniques I have been using that are associated with video microscopy and image analysis.
- When investigating the rheology of bacterial suspensions, we adopted a homemade microfluidic viscometer device. Details of the fabrication are shown in Sec. 2.3.
- A light-powered *E. coli* strain is used in the giant number fluctuations study and the emergence of active turbulence study (Chap. 4 and Chap. 5). This special

strain was obtained by transforming a wild-type strain with an exogenic plasmid which encodes a light-harvesting membrane protein. The discovery and working principles of the light-powering feature has been well documented by earlier works [86, 87, 88, 89, 90]. Following these works, I constructed a plasmid containing the gene and successfully transformed the wild-type *E. coli* strain. In Sec. 2.4, I will present the details on the materials and procedures I used to construct the mutant as a practical guide to those who need to further modify or trouble shoot the strain I made.

2.1 Motile Bacteria Sample Preparation

Peritrichous *E. coli* bacteria have been widely used as model micro-swimmers for active fluid studies [91, 92]. By bundling and unbundling their flagella, they achieve a so called “run-and-tumble” motion, allowing them to more efficiently explore their surrounding environment and to search for supplies. Fig. 2.1a shows a simplified model of a swimming *E. coli* bacterium model with a 2 μm rod-shape body and a helical-shape flagellum of around 8 μm . When swimming, all the flagella bundle together behind the cell and propel it forward [93]. Fig. 2.1b-c show the bundled state and unbundled state of the flagella, respectively. A swimming *E. coli* bacterium can generate nontrivial fluid flow, which can lead to hydrodynamic attraction to boundaries, alignment with other bacteria and other consequences [94]. It had long been assumed in theoretical works that the effective flow generated by microswimmers like *E. coli* is dipolar, with one force pushing forward from the head and another force pushing backward from the flagella [15, 95, 79, 80]. This assumption was then experimentally verified by Drescher et al. in 2011 [96], by reconstructing the flow field from many tracer particle trajectories. Fig. 2.1d-e show the flow field they measured and the best-fit force dipole flow. As I will show later, the swimming-induced flow plays a key role in the novel properties and

collective motions in the bacterial active fluids.

There are quite a few research groups over the world that are using *E. coli* suspensions to study active fluids. To name a few, Yodh and Arratia at University of Pennsylvania, Wu at Cornell University, Poon at the University of Edinburgh and Clement at ESPCI all have published experimental works using *E. coli* [59, 32, 62, 98, 60]. Although the protocols of preparing motile *E. coli* samples are similar across different groups' protocols, they have subtle differences from each other, which may be attributed to the specific strain of *E. coli*, ingredients of media and specific instrument conditions. Schwarz-Linek et al. proposed a sample preparation protocol based on standard bio-science manuals [99] and Berg's *E. coli* protocol. If one wants to learn how to prepare motile *E. coli* from scratch, it is recommended that he/she follows the protocol in Ref. [92].

When I joined the Cheng group at the University of Minnesota in 2015, before Ref. [92] was published, there was already a protocol in our lab that worked pretty well for us. I learned the protocol, and have made some modifications over the years to include the additional procedures for preparing light-powered *E. coli* and to optimize the motility and concentration of samples. Below I describe the protocol that works the best in Cheng lab.

2.1.1 Background Information

Bacterial strains We primarily work on two *E. coli* strains: *AW804* and *BW25113*.

AW804 is light-sensitive. *BW25113* is a wild type strain carrying a plasmid encoding green fluorescence protein, thus it is used when fluorescence / confocal microscopy is needed. Both strains have ampicillin resistance marker and thus require supplementing ampicillin to culturing media.

Antibiotics Bacteria are ubiquitous in the environment and can easily contaminate our

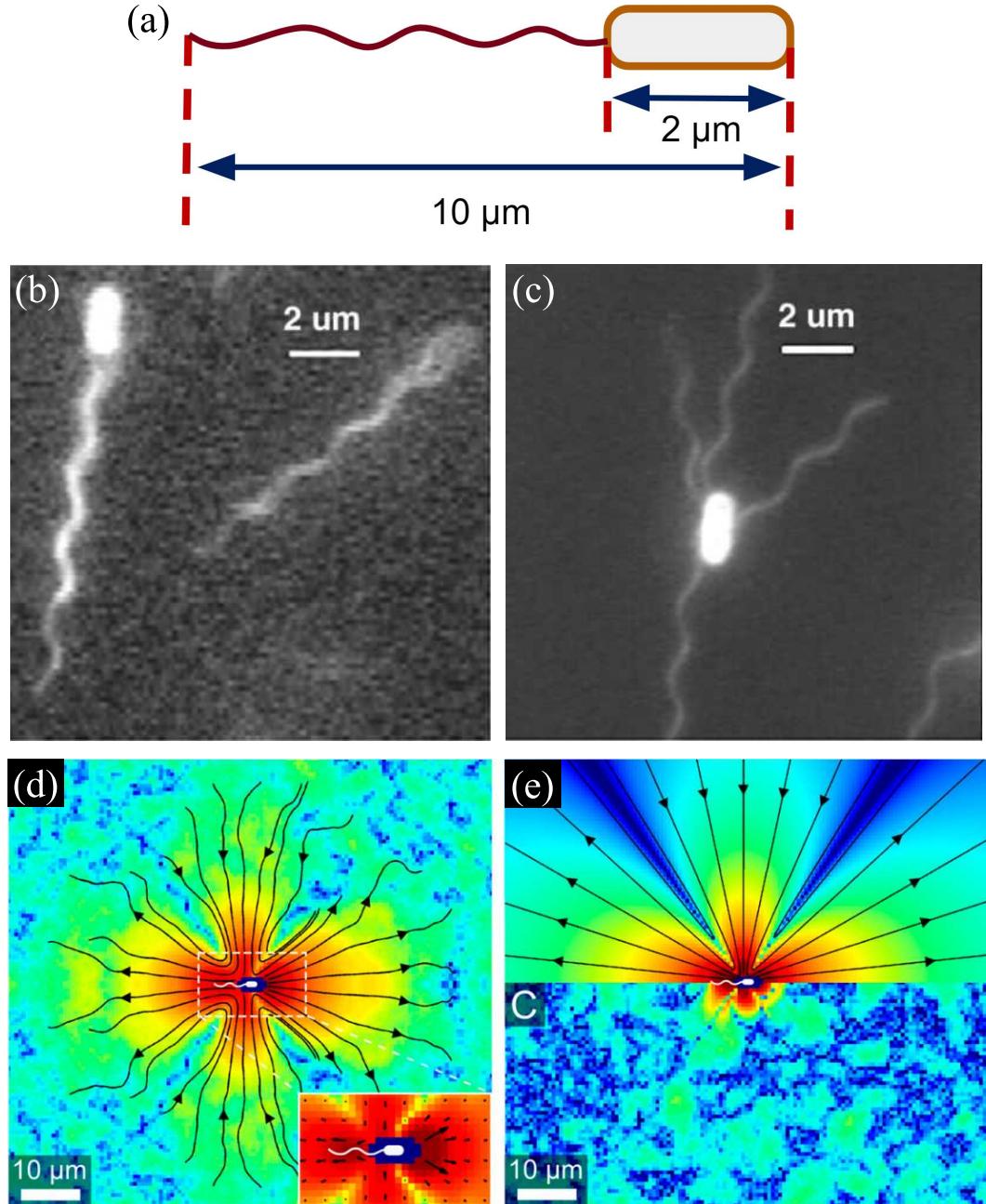


Figure 2.1: Model swimmer *Escherichia coli* and its flow field. (a) A schematic of a swimming *E. coli* bacterium. (b) Fluorescence microscopic image of swimming *E. coli* with bundled flagella. (c) Fluorescence microscopic image of tumbling *E. coli* with unbundled flagella. (d) Flow field around a swimming *E. coli*, measured with suspending microspheres. (e) Best-fit force dipole flow for the flow field shown in (d), overlaid on a fluorescence image of the bacterium. Image sources: (b) and (c) are reproduced from Fig. 4a and 2a in Ref. [97] with permission from XXX. (d) and (e) are reproduced from Fig. 1a and 1b in Ref. [96] with permission from XXX.

bacterial culture. In order to ensure the fidelity of the culture, we add antibiotic resistance markers to the bacteria we want to grow and meanwhile add antibiotics to the medium. The antibiotics inhibit the growth of contaminating species and allow our desired bacteria to grow normally.

Medium Various types of media (terrific broth, Luria broth, 2XYT and M9, etc.) are commonly used for bacterial culture. We use terrific broth. The recipe can be found in the protocol section.

2.1.2 Protocol

1. Prepare a 2-ml *E. coli* overnight culture.
 - (a) Prepare liquid terrific broth (TB). For example, to make 1 L TB, weigh out the following into a 1 L glass bottle:
 - 23.6 g Yeast extract (Sigma-Aldrich)
 - 11.8 g Tryptone plus (Sigma-Aldrich)
 - 4 ml Glycerol (XXX)
 - Add dI water to 1 LLoosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) and then loosely cover the top of the bottle with autoclave tape (stick cap and bottle body together to avoid cap popping off). Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the TB at room temperature.
 - (b) Using a sterile 10 ml pipette, transfer 2 ml TB to a sterile glass test tube.
 - (c) Using a sterile pipette, add 2 microliter (0.1% v/v) antibiotic solution to the TB in test tube.

- (d) Using a sterile pipette tip, pick a small chunk from our bacterial frozen stock (stored in the -80 °C freezer in 251) and carefully transfer the small chunk into the liquid TB + antibiotic.
- (e) Loosely cover the culture with sterile cap that is not air tight.
- (f) Incubate bacterial culture at 37 °C for 12-18 h in a shaking incubator.
- (g) After incubation, check growth, which is characterized by a cloudy haze in the media. This is the overnight culture.
2. Dilute overnight culture and harvest motile bacteria at mid-late log phase.
- (a) Using a sterile 10 ml pipette, transfer 3 ml TB to a sterile glass test tube.
- (b) Using a sterile pipette, add 2 microliter (0.1% v/v) antibiotic solution to the TB in test tube.
- (c) Transfer 30 microliter (1% v/v) overnight culture into the liquid TB + antibiotic.
- (d) Incubate bacterial culture at 30 °C for 6-6.5 h in a shaking incubator.
- (e) After incubation, check for growth, which is characterized by a cloudy haze in the media. This is the log phase bacteria.
3. Centrifuge for better motility and higher concentration bacterial sample.
- (a) Prepare motility buffer (MB), the following recipe is from Ref. [29].
- 0.01 M potassium phosphate (combine monobasic and dibasic solutions, Sigma-Aldrich)
 - 10^{-4} M EDTA (Sigma-Aldrich)
 - 0.002% weight fraction Tween 20 (Sigma-Aldrich)
 - Adjust pH to 7.0

- (b) Take out the log phase bacteria from the shaking incubator, centrifuge for 5 min at 800 rcf.
- (c) Discard the supernatant quickly and transfer the left-over liquid to a new centrifuge tube.
- (d) Add 500-1000 ul MB (or water) to resuspend the bottom pellet (avoid bottom pellet) and centrifuge for a second time (5 min, 800 rcf).
- (e) Discard the supernatant and let the tubes sit for two minutes. The remaining left-over liquid should be now filled with the active *E. coli*. Take the left-over solution in another capsule and use it for experiments.
- (f) To measure the concentration, transfer 10 microliter of the suspension into a 1 ml plastic cuvette and dilute 100 times (by adding 990 microliter water). Put the cuvette in the spectrophotometer in 251 and use the OD600 program. The resulting number times 100 will be the number density of your suspension in the unit of n_0 (8×10^8 cells/ml).

2.2 Video Microscopy and Image Analysis

In my experimental research, a standard workflow is

- Take videos of samples such as swimming bacteria
- Analyze the videos, typically extracting particle position and velocity information from the videos
- Calculate from the position and velocity information to obtain more complex information, such as flow field, kinetic energy and diffusivity

From this workflow, one can tell that the video microscopy and image analysis are the core skills that enable me to conduct the research. In this section, I will decribe how I

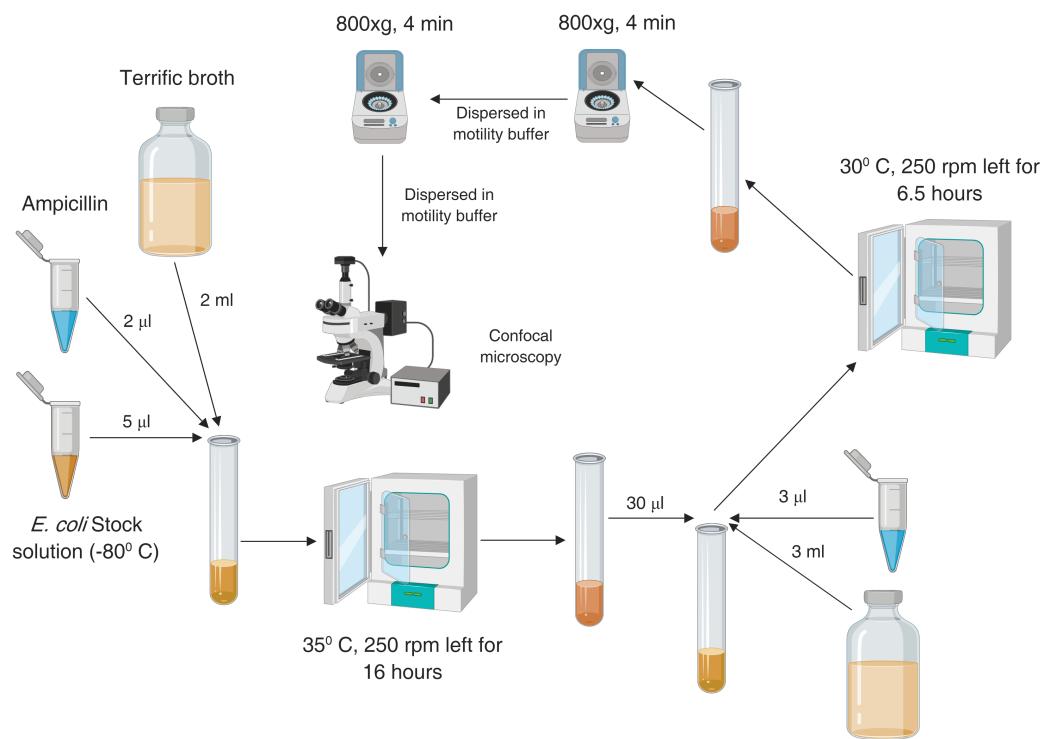


Figure 2.2: Graphical motile *E. coli* sample preparation protocol. Image courtesy of Shashank Kamdar.

overcome practical challenges when applying these skills in experiments. I want to note that the standard manuals are always the best reference for beginners who have just started to learn about a technique. In my case, the standard manuals are the Nikon inverted microscope Eclipse Ti-E Ti-E/B instructions [100], OpenPIV official website [101, 102] and trackpy official website [103]. Some related projects (listed in the websites mentioned) also provide valuable tutorials and ideas, for example the particle tracking routines in IDL by Crocker and Weeks [104] and in Matlab by Blair and Dufresne [105].

2.2.1 Video Microscopy

Power *E. coli* with Illumination Light

In the studies of the giant number fluctuations and the emergence of active turbulence, I used a light-powered *E. coli* mutant, which changes its swimming speed according to the amount of light it receives (details of the light-powered *E. coli* mutant can be found in Sec. 2.4).

I use the illumination light of the microscope as the power source of the bacteria, instead of using another light source, based on two considerations: 1) an additional light source shining on the sample will lead to additional unexpected light going into the objective, which often leads to bad image quality; 2) It is hard to construct a spatially uniform light, especially when it has to come in an angle not perpendicular to the specimen. Therefore, I use the illumination light of the microscope to power the *E. coli*.

The light-powered *E. coli* mutant requires quite a high light intensity to move fast enough. Such a high intensity cannot be achieved in the normal microscopy conditions, where four light filters are applied for different purposes. Fig. 2.3a-b show the Nikon Ti-E inverted microscope and the illumination light filtering system with the four light filters designated as ND, D, NCB and PFS. The functions of the filters are listed in

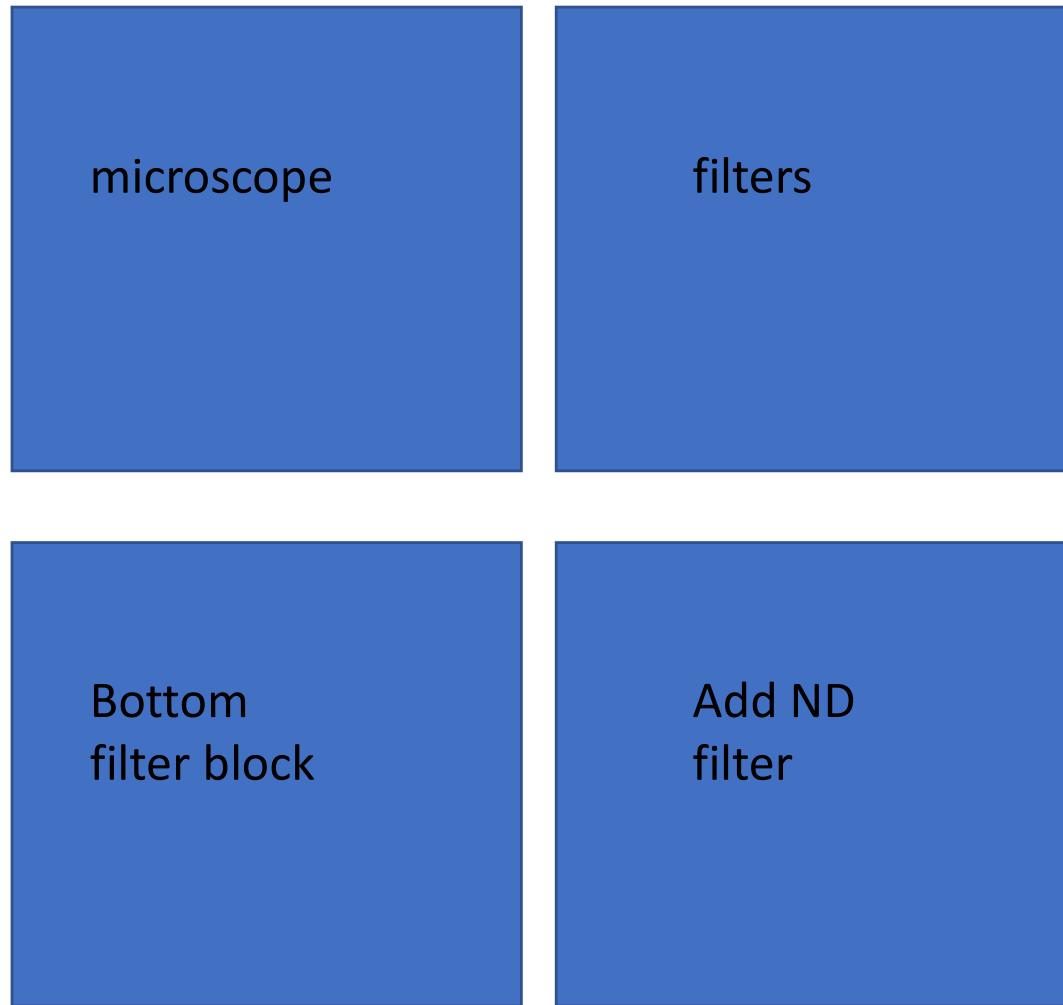


Figure 2.3: **Nikon Ti-E inverted microscope and its filters.** (a) Nikon Ti-E inverted microscope model. (b) Illumination light path filters. (c) Filter block under objective. (d) Adding additional ND filter under objective.

Table. 2.1:

ND	Neutral density filter: adjust the brightness for normal microscopy or photomicroscopy
D	Diffusion filter: made of frosted glass and will diffuse light, used for equalizing the illumination
NCB	Neutral color balance: corrects the color temperature for mnormal microscopy or filming by daylight type color. Note: this filter is essential for optimal color reproducibility when taking color images, and it should be kept out of the optical path when filming in black and white.
PFS	Perfect focusing system: a hardware solution to combat axial focus fluctuations in real time during long-term imaging investigations. Note - this filter should be kept out if one does not intend to use the perfect focusing system.

Table 2.1: Filters in the illumination light path and their functions.

Removing some of those filters can make the illumination light strong enough to power the bacteria. According to the functions of the filters, the only necessary filter is the diffusion filter, given that one is not doing color imaging and is not using perfect focusing system, as it is in my experiment. Fig. 2.4a shows an image taken without the diffusion filter (D). Without diffusing the illumination light, the resulting image is clearly inhomogeneous in a large range, with a bright center and a dark bottom area. By putting the diffusion filter in the illumination light path, one can make the illumination light much more uniform, as shown in Fig. 2.4b. All the other filters (ND, NCB, PFS) are effectively reducing the overall intensity. Putting in or out these filters only results in globally dimmer or brighter images, without changing the detailed patterns in the image. Thus, these three filters are optional in my experiment. Since powering the light-powered *E. coli* requires a very high light intensity, only the diffusion filter should be kept in the illumination light path.

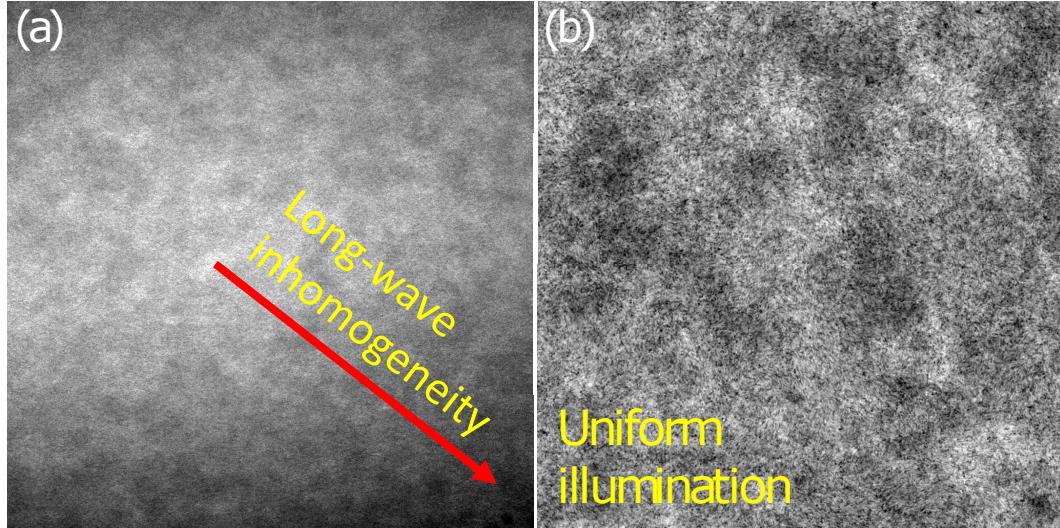


Figure 2.4: Image with (a) and without (b) the diffusion filter (D).

Avoid over exposure

While I have achieved high enough light intensity to power the bacteria, another problem occurs - the light is so strong that the camera is over exposed. In order to power the bacteria, the illumination light intensity cannot be reduced. The only way to avoid the over exposure is to add an filter between the specimen and the camera. What I did is shown in Fig. 2.3c-d. I took out one of the filter cube from the turret under the objective, which is originally used for fluorescence microscopy. Then I put a piece of neutral density filter on top of the cube and put the cube back to the turret. This additional filter allows for the imaging under strong illumination light.

2.2.2 Image Analysis

With the fast developments of digital imaging, human are enabled to investigate many processes, ranging from astronomical object motions to microorganism behavior, in unprecedented detail [106]. While it is getting easier than ever to acquire large amount

of images, a demand for automated image analysis has also become unprecedented [107, 108, 109].

Particle tracking has been one of the most useful automated image analysis tools in the study of colloids and microorganisms. Over the last 20 years, it has developed significantly and many algorithms, toolkits and all-in-one softwares have been implemented and applied in a variety of image analysis tasks. Despite the abundance of particle tracking tools, no agreement has been made on which one in the vast collection of tools works best. Much effort has been devoted to answer this question by comparing the performance of different tools [106, 110, 111, 112, 113, 114, 115, 116]. In these studies, though different algorithms show different performance, no single algorithm outperforms all the others in all scenarios.

Particle tracking is generally composed of two steps: particle detection (spatial) and linking trajectories (temporal). For the detection step, based on the feature (generalized “particle”) shape sought, different methods are used. For point features, a local maxima finding method is often used; for edge features, group labeling is often used; and for region features, region seeding is often used [110].

Anisotropic Particle Tracking

A challenge I encountered when working on the diffusion project was the detecting of the ellipsoidal particles surrounded by moving microorganisms (bacteria and algae). A local maxima finding method was applied previously to detect the center of an ellipsoidal particle [63]. Combined with intensity fitting around the center in different directions, the orientation of the particle can also be obtained. The same method, however, does not work well for my image because of the presence of microorganisms, which give rise to many more local maxima in the image (a typical image is shown in Fig. 2.5a). To overcome this challenge, I adapted a thresholding and connected group labeling based

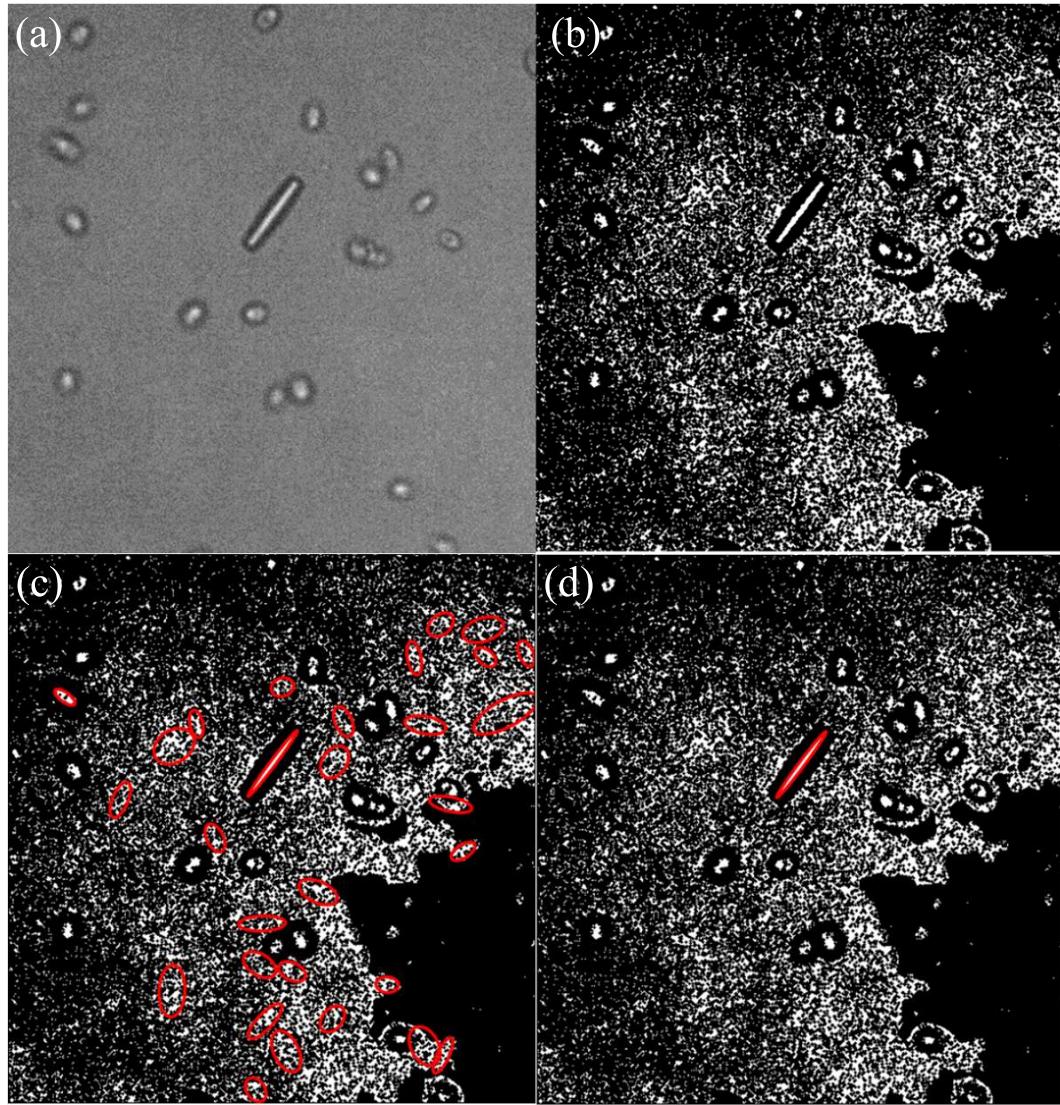


Figure 2.5: Illustration of ellipsoidal particle detection by thresholding and group labeling. (a) The raw image: an ellipsoidal polystyrene particle surrounded by swimming algae in a suspension. (b) Binarized raw image. (c) Detection result of connected white regions. (d) Final result after appropriate filtering.

method from Ref. [117, 118, 119]. The method has the following steps:

- threshold the grayscale image (Fig. 2.5a) to a binary image (Fig. 2.5b)

- find connected white regions (`skimage.measure.label` in Python)
- find the best-fit ellipse of each white connected regions found in the last step, as indicated by the red ellipses in Fig. 2.5c (`skimage.measure.regionprops` in Python)
- filter the parameters of the ellipses with appropriate criteria, so that only the desired ellipse is found, as indicated by the red ellipse in Fig. 2.5d

Fig. 2.5 only illustrates the essential idea of the method. When applying the method, some necessary image preprocessing, including linear, nonlinear and frequency filters need to be used to make sure the desired features stand out, and the undesired noises are suppressed. The preprocessing techniques have been reviewed in Ref. [110, 109]. This method was used to obtain the trajectories of ellipsoidal particles in algal suspensions, which was then used to calculate the diffusivity for my first collaborative project, reported in Ref. [34].

Manual Particle Tracking Software: *manTrack*

Despite the great advances of particle tracking techniques, the accuracy suffers much from poor image quality and mutual touching of objects in images, especially when imaging dense suspensions of microorganisms [111]. A very challenging particle detecting task is manifested by a dense suspension of collectively moving bacteria, as shown in Fig. 2.6a. Despite the use of confocal microscopy, most bacteria are seen to be overlapping with others and are in very different shapes, making it impossible for existing tools to detect all the desired features. In a scenario like this, human eyes are the most powerful complementary tool to machines.

Manually marking the positions of particles is feasible when one image only contains one or several particles. When particle numbers get large, however, it is no longer

feasible (a typical video I take contains hundreds of particles). I combined the automated tracking and the manual tracking together in order to take the advantages of both: fast and reliable, by implementing a manual tracking software *manTrack* with graphical user interface (GUI). The workflow for using *manTrack* is:

- Use an automated method to do a preliminary particle tracking (or detection).
- Load the preliminary automated detection result into *manTrack*. The result will be displayed in *manTrack* GUI as elliptical contours, as shown in Fig. 2.6b.
- When “delete mode” is enabled, one can delete one entry from the result by clicking on the elliptical contour. In Fig. 2.6c, the entry at the tip of the red arrow has been deleted.
- When “track mode” is enabled, one can add an entry to the current result by drawing an ellipse in the image, as shown by the black contour in Fig. 2.6d.

manTrack provides a user friendly GUI for efficient modification of preliminary automated particle detection results. The combination of machine automated detection and human eye detection makes possible good accuracy and an acceptable execution speed. This software was used for analyzing the orientation of bacteria in active turbulence (Chap. 5).

Several new tools have been developed in recent years, such as *TrackMate* and *tTt* [120, 116]. The fast growth of technologies in other fields, especially artificial intelligence, has stimulate the development of new tracking techniques [121, 122].

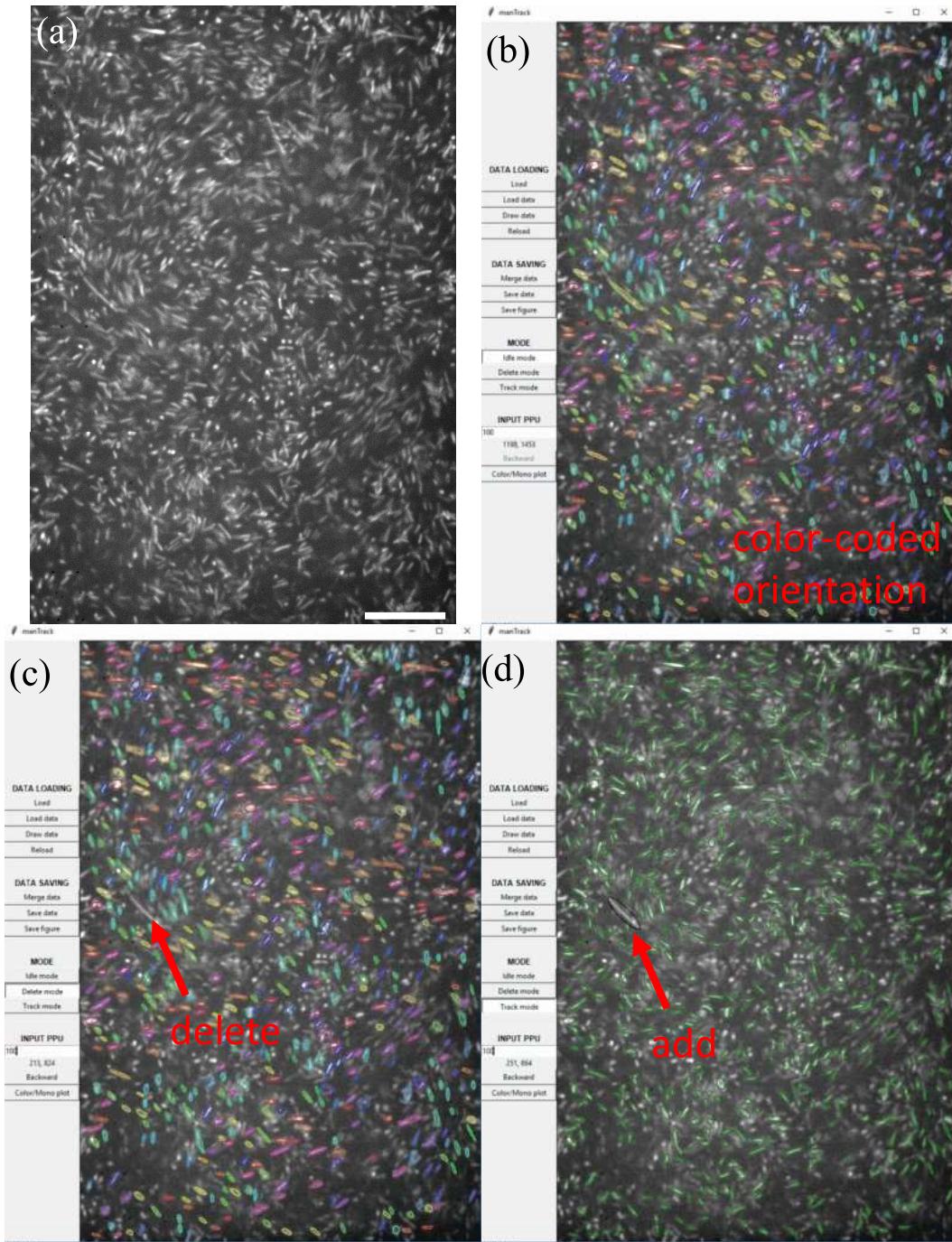


Figure 2.6: **A challenging particle detection task and the manual tracking software.** (a) Confocal microscopy image of *E. coli* collective motion ($\phi = 6.4\%$). (b)-(d) Snapshots of the manTrack software, demonstrating color-coded orientation, manual deleting and manual adding.

2.3 Micro-fabrication and Microfluidics

2.4 Light-controlled E. coli: Genetic Modification, Culturing and Trouble Shooting

Chapter 3

Rheology of Bacterial Suspensions under Confinement*

3.1 Introduction

3.2 Methods

3.3 Results

3.4 Discussion and Conclusion

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Chapter 4

Giant Number Fluctuations in 3-Dimensional Space

4.1 Introduction

4.2 Methods

4.3 Results

4.4 Discussion and Conclusion

Chapter 5

The Emergence of Active Turbulence*

5.1 Introduction

5.2 Methods

5.3 Results

5.4 Discussion and Conclusion

*Reproduced in part with permission from (Yi Peng, Zhengyang Liu and Xiang Cheng, “Imaging the emergence of bacterial turbulence using light-powered *Escherichia coli*”, *arXiv e-print*).

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