

Three-dimensional measurements of dynamics and structure in kinesin-driven active fluids

by

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Vitae

Yi Fan was born March, 1989 in Sichuan Province and raised in Hubei Province, China. She attended Xi'an Jiaotong University, from which she graduated with a bachelor's degree in Mechanical Engineering in 2011. She completed her master's degree in Engineering at Brown University in 2013 and then moved to University of Sydney as a visiting research scholar. In 2014, she returned to Brown as a PhD student and completed her doctorate degree at School of Engineering in Brown University in 2018.

Preface

The research described herein was conducted under the supervision of Professor Kenneth S. Breuer at the School of Engineering, Brown University, between October 2014 and May 2018. It contains experimental studies of kinesin-driven active fluids subject to various geometrical confinements.

The content is as follows and includes the synthesis of several manuscripts:

Introduction. A general description of active matter research topics and an introduction of the artificial active fluids associated with following experiments.

“Simultaneous two-color fluorescence microscopy with three-dimensional particle tracking velocimetry” by Y. Fan, Z. Qu and K. S. Breuer. This work is primarily supported by NSF-MRSEC-1420382. We also acknowledge the facilities supported by NSF-1336638. Y. Fan acquired data in cytoskeletal purification; Z. Qu and Y. Fan acquired data in bacterial suspension; Y. Fan, Z. Qu and K. S. Breuer designed the experiment.

“Three-dimensional structure of coherent flows in kinesin-driven active fluids” by Y. Fan, K.-T. Wu, S. Fraden, Z. Dogic and K. S. Breuer. This work is primarily supported by NSF-MRSEC-1420382. We acknowledge the use of facilities supported by NSF-1336638. We acknowledge the use of facilities in Institute for Molecular and Nanoscale Innovation at Brown University and Materials Research Science and

Engineering Center at Brandeis University. Y. Fan performed the experiment and analyzed the data; Y. Fan and K.-T. Wu designed the experiment; Y. Fan, S. Fraden, Z. Dogic and K. S. Breuer designed the research.

“The loss of isotropy due to confinement in kinesin-driven active fluids” by Y. Fan, K.-T. Wu, A. Aghvami, S. Fraden, Z. Dogic and K. S. Breuer. This work is primarily supported by NSF-MRSEC-1420382. We acknowledge the use of facilities in Materials Research Science and Engineering Center at Brandeis University. Analysis of data was supported by Oscar computing cluster in Center for Computation and Visualization at Brown University. Y. Fan performed the experiment and analyzed the data; Y. Fan, K.-T. Wu and A. Aghvami designed the experiment; Y. Fan, S. Fraden, Z. Dogic and K. S. Breuer designed the research.

Interaction between active microtubules and passive particles. This chapter includes experimental study of small-scale interaction between active microtubules and nearby passive particles.

Conclusion. Final remarks and recommendations for future work.

This work is to the best of my knowledge original, except where acknowledgements and references are made. Neither this, nor any substantially similar dissertation has been or is being submitted for any other degree, diploma or other qualification at any other university.

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CHAPTER ONE

Introduction

Active matter defines a broad category of self-driven materials which share a unifying characteristic of converting kinetic energy generated at small scales into coherent motion at larger scales. Although the definition of active matter has just come to be known during the past few decades [1, 2, 3], scientific research can be traced back to the study about flocking behavior of birds in the 1930s [4]. In the language used by active matter researchers, a flock includes a collection of self-propelled agents, each of which continuously injects energy at small scales to the overall system. Therefore, active matter does not follow the principles of equilibrium statistical mechanics and exhibits dynamical states that are far from equilibrium [5].

The study of active matter covers a wide range of topics. The physics of flocking remains an active topic since the 1930s and has expanded from birds [4, 6] to other diversity, such as penguins [7], fish [8], insects [9], cells [10, 11] and motile bacteria [12]. It covers living creatures powered by internal active stress from individuals, and non-living self-driven particles powered by external active stress [13, 14, 15]. In the 1980s, theoretical work [16, 17], also inspired by the natural flocking behavior of birds, started to animate dynamics observed in active matter and continued to explore fundamental rules that govern such systems [18]. In the meanwhile, more sub-cellular processes and their functionalities were identified [19, 20, 21, 22] in biology and chemistry, which built the foundation of artificial biological active materials. These systems, which can be assembled hierarchically in a research lab with precise control over each constituent [23, 24, 25], provide insight towards understanding micro-biological phenomena observed in nature and further inspire scientists in assembling synthetic artificial machines with life-like properties [26].

In this thesis, we study a specific artificial system first reported by Sanchez and his colleagues in 2012 [27]. This system is comprised of microtubule filaments, polymerized from tubulin dimers, with an average length of about $1\ \mu\text{m}$ [28]. Polymer

micelles (pluronic F 127) in the aqueous solvent [29] induce bundling of the microtubules through volume exclusion, which generates a depletion force. Kinesin motors, attached to streptavidin tetramers, bind to and walk along neighboring microtubules with each motor progressing towards the plus end of its associated microtubule filament [30, 31, 32]. Between a pair of antipolar microtubule filaments, the motors generate a sliding force and, as a consequence, the bundled microtubule network exhibits an extensile behavior that collectively results in a writhing dynamical state in which the microtubule network extends, buckles, folds and reforms in a continuously reconfiguring chaotic, almost turbulent-like system [27, 33].

The dynamics of these active systems has been extensively quantified by varying concentrations of the biological constituents [27, 34]. In addition, in a confined environment, the active systems exhibit at least two different dynamical states [28]. In one state, the system demonstrates a chaotic turbulent character, apparently without any preferred direction or orientation; in a second state, a preferred direction emerges on top of the turbulent-like dynamics [28]. However, the transition between these two dynamical states and the fundamental principles that govern each state remain unclear. Besides, previous work [28] has only measured the motion in a single slice two-dimensional plane and so the three-dimensional structure of these active microtubule networks remains un-explored.

Here, we develop techniques to measure the three-dimensional structure of these microtubule systems, and study both the chaotic and advective dynamical states of the active systems, their statistics and corresponding flow structure in three dimensions, and the way in which these properties vary as we change the confining geometries.

CHAPTER TWO

Simultaneous two-color fluorescence microscopy with three-dimensional particle tracking velocimetry

2.1 Introduction

It is important to review on target testing materials when designing an experimental technique. Active matter, as the target testing sample, consists of active agents that are individually capable of converting small-scale energy into overall motion of the system [5, 26]. Active agents interact with surrounding media, drive the system out of equilibrium and induce flocking behavior. In various types of active matter, individual interaction and flocking physics are studied via direct observation or localization of active agents, including live individuals (birds [6], fishes [8] and bacteria [12]), synthetic biological materials (cytoskeletal extracts [35, 36, 37, 38]), and nonliving materials (self-propelled rods or particles [13, 14, 15]). Another approach of measurement is using dispersed passive tracer particle and performing two-dimensional particle tracking velocimetry (PTV) or particle image velocimetry (PIV), to quantify dynamics at microscopic scale, such as in bacteria suspensions [39, 40, 41] and in cytoskeletal extracts [27, 28]. Although current research well quantifies the active individuals and the passive flows in the system, there are many remaining questions. For example, what is the three-dimensional dynamics of an active system? How passive tracer particles interact with active agents? The design of an experimental set-up introduced here is inspired by these questions. It allows simultaneous observation of active agents and passive particles and provides three-dimensional measurements using z -scanning and off-focus particle tracking [42, 43, 44, 45].

The experimental set-up, with modest adjustments, can be used to investigate different active matter systems at microscopic scale. However, in this thesis, we focus on a specific assembled biological system, consisting of kinesin-streptavidin motor clusters, microtubule filaments (fluorescently-labeled, red emission wavelength), and polymer micelles, as presented in Figure 2.1. The polymer micelles apply depletion

force to induce bundling of microtubule filaments through volume exclusion. The motor clusters generate local active stress in bundled microtubules, by converting chemical energy from adenosine triphosphate (ATP) into inter-filament sliding motion [20, 27, 31]. The local active stress drives the system out of equilibrium, and the active flows are measured using embedded fluorescent tracer particles (blue emission wavelength). In following sections, we refer the biological system as “active fluids” to reveal the predominant liquid-like property.

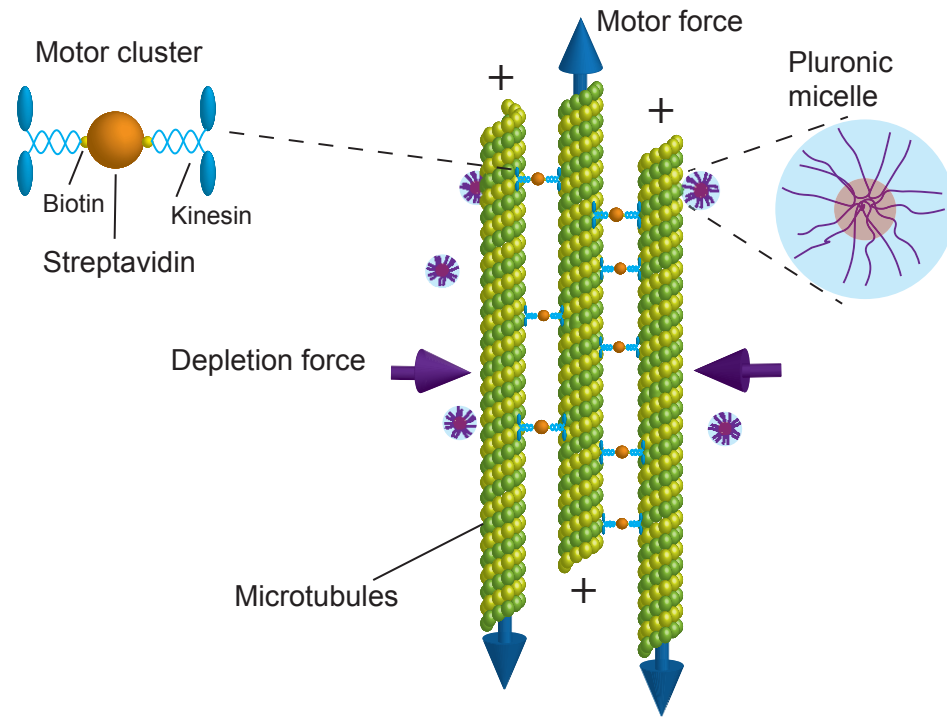


Figure 2.1: A sketch illustrating the basic assembling unit of the cytoskeletal purification. The depletion agents (pluronic micelles) force microtubule filaments to bundle by excluding fluid. It enables clustered kinesin motors to generate sliding force by walking towards plus ends between neighbor microtubules with anti-polarity. The bundle shows extensile behavior in the presence of chemical energy.

2.2 Experimental set-up

To build an experimental set-up with two-color imaging and three-dimensional scanning (Figure 2.2), following equipment were added to an epi-fluorescence microscope (Nikon TE 200 eclipse): a four-wavelength high power LED source (Thorlabs, DC4100), two-bandwidth optical components, an air immersion objective (Nikon CFI Plan Apo VC 20 $\times$, WD = 1 mm, NA = 0.75 or Nikon CFI S Plan Fluor ELWD 20 $\times$, WD = 6.9, NA = 0.45) held by a piezo z-drive (Physik Instrumente, Model P-725), an optical splitter (Cairn Research, Optosplit II) and an sCMOS camera (PCO edge 5.5). The microscope and associated equipment were contained inside a chamber which maintained the temperature at 25°C $\pm$ 0.3 using an air-therm heater (World Precision Instruments, Airtherm-sat-1W).

Two LEDs (405 nm and 565 nm) of the LED source were energized for sample illumination. The excitation light, refined using a two-bandwidth excitation filter (Chroma Technology, 59003x, 395/30 nm and 575/30 nm), is directed through the air immersion objective held by the piezo z-drive and a cover (microscope glass cover slide or COC film) to the active sample. The fluorescently-labelled microtubules (Thermal Fisher Scientific, A20003, excitation/emission: 578 nm/603 nm) and fluorescent tracer particles (Fluoro-Max Blue, Thermal Fisher Scientific, B0100, excitation/emission: 412 nm/447 nm, or Purple plum, Bangs Laboratories, FS05F, excitation/emission: 395 nm/428 nm) in the active sample emitted light at red and blue wavelengths respectively. The emission light passed through a two-bandwidth dichroic mirror (Chroma Technology, 59003bs, transmission in 460/30 nm and 630/60 nm) and was directed to an optical splitter that separated the two colors using a dichroic mirror (Chroma Technology, 565dcxr). The light streams passed through corresponding band-pass filter (Chroma Technology, ET450/50m and D620/60m) in

the optical splitter before being projected onto two halves of the sCMOS sensor. Although the full sensor size of the camera is $2560 \text{ pixels} \times 2160 \text{ pixels}$, limitations with the windows of the optical splitter resulted in a final image of $2500 \text{ pixels} \times 1300 \text{ pixels}$. The camera settings for image recording and writing will be discussed in each chapter with experimental details.

The three-dimensional scanning was accomplished by the piezo z-drive, which can hold the objective and scan up to $400 \text{ }\mu\text{m}$ along the z -axis. The z-drive accepts a voltage input between $[0, 10] \text{ V}$, where 0 represents the home position and 10 V represents the maximum position ($400 \text{ }\mu\text{m}$ above); other positions are linearly related to the input voltage. During each experiment, the scanning positions were provided by a controlling computer, using a digital-to-analog converter (National Instruments, DAQ PCI6052e). For the purpose of synchronization, equipment were configured in a manner that the LED and the camera worked while the z-drive was stationary, or vice versa, the LED and the camera recessed while the z-drive was moving and settling. All signals of synchronized equipment, the target scanning positions and the actual scanning positions were monitored using an oscilloscope (Tektronix, DPO 2024B). The determination of target scanning positions requires experimental details about image recording frequency, geometry of tested channels and the settling time of the piezo z-drive. Therefore, it will be covered in each chapter.

2.3 Distortion correction and two-color registration

Each optical component in the microscope and in the optical splitter introduces distortions which require two stages of calibration: (i) correction in each sub-frame

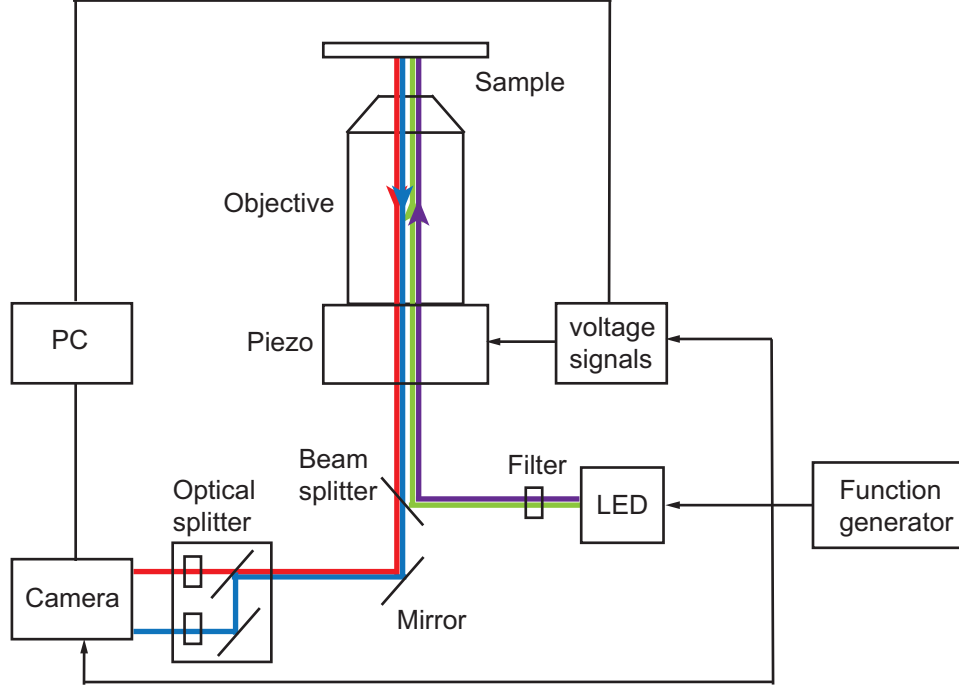


Figure 2.2: The experimental set-up in an epi-fluorescence microscope equipped with a piezo z -scanning drive and a simultaneous two-color fluorescent imaging system. The z -scanning and image recording are synchronized.

of the optical splitter and (ii) registration of the two sub-frames for the same physical observation region.

In the first stage, image corrections in both sub-frames of the optical splitter followed the same analytical model reported by Brown and Fryer [46, 47] and error estimations used the method illustrated by Soloff *et al.* [48]. Figure 2.3 shows the process of distortion correction among a true grid target, a distorted image (exaggerated) and a corrected image. In a grid target (Thorlabs, R1L3S3P) with $50\ \mu\text{m}$ square rulings, true locations of grids are known as (x_t, y_t) . After optical components in the microscope and the optical splitter, recorded image is distorted and locations of corners become (x_d, y_d) . We assume that the major distortion is originated from radial distortion and tangential distortion. The radial distortion, introduced by optical lenses, is zero at the optical center, (x_o, y_o) , and increases with radial distance, d , where $d = \sqrt{(x_d - x_o)^2 + (y_d - y_o)^2}$. The radial distortion between true locations

and distorted locations in x -axis and in y -axis can be estimated by Taylor expansions:

$$\begin{aligned}x_t &= x_d(1 + k_1d^2 + k_2d^4), \\y_t &= y_d(1 + k_3d^2 + k_4d^4),\end{aligned}\tag{2.1}$$

where k_1 , k_2 , k_3 and k_4 are radial distortion constants. The tangential distortion between true locations and distorted locations, introduced during assembling of optics and cameras, can be estimated as

$$\begin{aligned}x_t - x_d &= 2p_1x_dy_d + p_2(d^2 + 2x_d^2), \\y_t - y_d &= 2p_3x_dy_d + p_4(d^2 + 2y_d^2),\end{aligned}\tag{2.2}$$

where p_1 , p_2 , p_3 and p_4 are tangential distortion constants. From Equation 2.1 and Equation 2.2, we can map distorted locations, (x_d, y_d) , to true locations, (x_t, y_t) , by two fifth-power functions:

$$\begin{aligned}x_t &= F(x_d^5, y_d^5, \dots, x_d, y_d, 1), \\y_t &= G(x_d^5, y_d^5, \dots, x_d, y_d, 1),\end{aligned}\tag{2.3}$$

where F and G are acquired distortion correction functions. They are used to calculate locations in the corrected image, (x_c, y_c) , as

$$\begin{aligned}x_c &= F(x_d^5, y_d^5, \dots, x_d, y_d, 1), \\y_c &= G(x_d^5, y_d^5, \dots, x_d, y_d, 1).\end{aligned}\tag{2.4}$$

We define distortion error as the difference between the distorted image and the

corrected image:

$$\epsilon_d = \sqrt{(x_d - x_c)^2 + (y_d - y_c)^2}, \quad (2.5)$$

and correction error as the difference between the corrected image and the true grid target:

$$\epsilon_c = \sqrt{(x_c - x_t)^2 + (y_c - y_t)^2}. \quad (2.6)$$

We repeated the same analysis in both sub-frames of the optical splitter. Figure 2.4 shows distortion errors from red and blue sub-frames. Along each contour, distortion error, ϵ_d , is equivalent. Figure 2.5 presents correction errors in red and blue sub-frames of the optical splitter.

In the second stage, corrected images, (x_c^r, y_c^r) (corrected image from red sub-frame) and (x_c^b, y_c^b) (corrected image from blue sub-frame), were registered by linear registration functions:

$$\begin{aligned} x_c^r &= mx_c^b + ny_c^b + q, \\ y_c^r &= m'x_c^b + n'y_c^b + q', \end{aligned} \quad (2.7)$$

where m , n , q and m' , n' , q' are mapping constants. Considering the fluorescence in active samples, registration functions located passive tracer particles to active microtubule bundles.

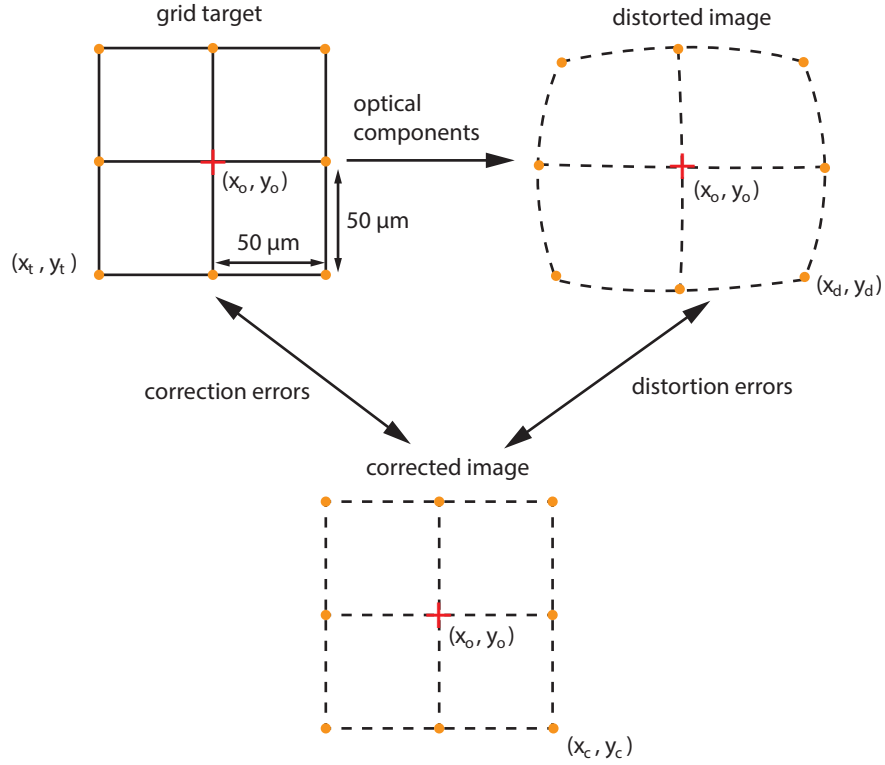


Figure 2.3: A sketch illustrating the process of image distortion correction based on a true grid target, a distorted image (exaggerated) and a corrected image. Distortion correction functions are generated from the grid target and the distorted image. The corrected image is calculated with the distorted image and distortion correction functions. Calibration error is estimated by distortion errors and correction errors.

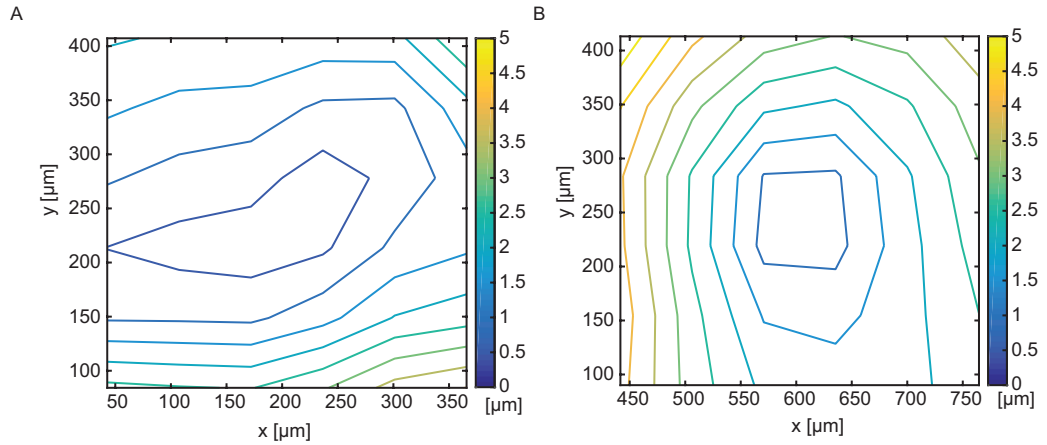


Figure 2.4: The distortion errors (Equation 2.5) in two sub-frames of the optical splitter: (A) “red” sub-frame and (B) “blue” sub-frame.

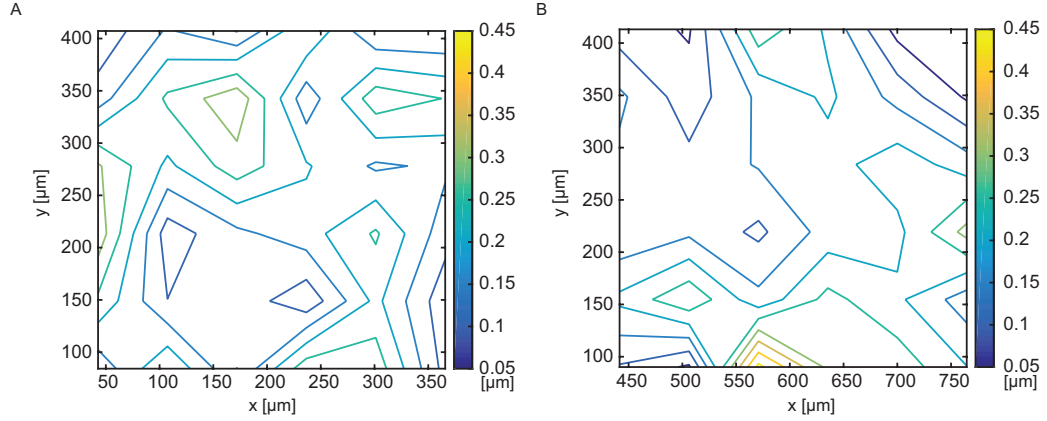


Figure 2.5: The correction errors (Equation 2.6) in two sub-frames of the optical splitter: (A) “red” sub-frame and (B) “blue” sub-frame.

2.4 Three-dimensional particle tracking velocimetry

2.4.1 Point spread function

In the observation volume, each excited particle acts as a point source of light. The emitted fluorescent light of the point is diffracted at the objective aperture, spreading the wave front into a diffraction pattern, known as a point spread function [49]. The image of the particle in the $x - y$ plane, parallel to the focal plane and perpendicular to the optical axis, appears as a ring or series of concentric rings, commonly referred to as the Airy disk, whose diameter depends on the distance of the particle from the focal plane [50]. Depending on the objective characteristics and the imaging mode, the Airy disk has a unique shape and size. The center of the Airy disk corresponds to the $x - y$ position of the tracer particle, while if one can determine the relationship between the Airy disk size and the distance from the focal plane, the locations of imaged tracer particles along z -axis can also be resolved. Therefore, three-dimensional particle detection is transformed into a problem of identifying the

center and radius of the imaged circles [45, 51].

2.4.2 Circle detection algorithm

In a typical group of experiments, images that require post-processing contain a pixel quantity at an order of 10^{12} or even higher. The circle detection procedure, as the most time-consuming part in computation, is carefully balanced between processing speed and observation volume. Because a larger observation volume, demanding detection of more particles with a larger range of radii, slows down the speed of image processing, whereas a small observation volume restricts statistics with less amount of detected particles.

The circle detection algorithm used is a well-developed method called the Circle Hough Transform (CHT) [52]. The detection code follows fundamental pattern recognition concepts [53], CHT algorithm and parallel processing method in MATLAB Image Processing Toolbox. Besides, we modify the code based on a recent improvement in detection of overlapped circles [45]. The key procedures are illustrated in Figure 2.6 as:

1. The raw image is pre-processed using Gaussian filtering (Figure 2.6A) to smooth image contrast and suppress inner concentric rings in the Airy disk.
2. A Sobel operator is used to perform ridge detection [45]. The Sobel operator, as a first-derivative-based operator, has a smaller convolution matrix and is less expensive for computation. Different from edge detection [54], which detects the highest contrast between dark and bright regions, ridge detection identifies the highest intensity in the neighborhood so that the recognized ridge remains

on the outer ring of the Airy disk. Detected ridges include outer fringes of the Airy disks, edges of in-focus particles and noises.

3. A threshold is applied to all detected ridges to transform the image into a binary image (Figure 2.6B).
4. The binary image with detected ridges and a target range of radii are input for “voting” process of CHT. Each pixel on the ridge votes once for a radius, which is referred as one “voting round”. More radii in the target range means more voting rounds, which consume more time for processing. The highest vote simply occurs when the proper radius is selected. The pixel location which receives the highest vote, is the circular center. In an image frame with multiple Airy disks, local maxima of intensity correspond to particle centers (Figure 2.6C and D).
5. The Pratt model [55] is used to perform least-squares fitting on detected circles (Figure 2.6E) so that sub-pixel accuracy is obtained (Figure 2.6F).

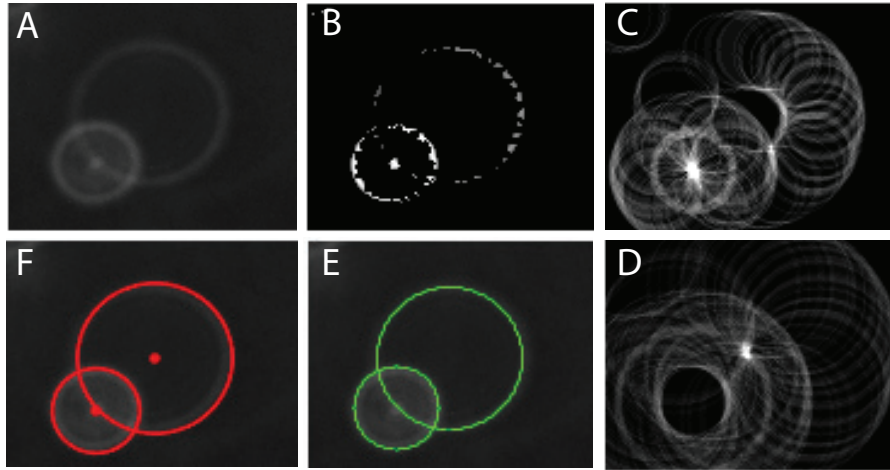


Figure 2.6: Main procedures of Circle Hough Transform for particle detection. (A) Pre-processed image after Gaussian filtering. (B) Ridge detection by a Sobel operator and image thresholding. (C) “Voting” process with a smaller target radius. The maximum vote corresponds to the center of the smaller diffraction pattern. (D) “Voting” process with a larger target radius. The maximum vote corresponds to the center of the larger Airy disk. (E) Detected particles. (F) Circle fittings on detected particles.

2.4.3 Position calculation

Figure 2.7 shows a stack of scanned images of an immobilized fluorescent particle (1 μm diameter particle) in 1% (w/w) agarose gel at a scanning step of 0.1 μm . A reference library is established from detected radius of the Airy disk to corresponding z -position (Δz distance below the focal plane), which follows a quadratic function (Figure 2.8). The in-focus particle (if the Airy disk radius less than 2 μm), which presents no significant variation in the radius, is not used for z -position calculation. We focus on the negative side of the point spread function (Figure 2.7, between $-5 \mu\text{m}$ to $-58 \mu\text{m}$) due to the asymmetric intensity of the Airy disk along the optical light path, parallel to z -axis. Similar to off-focus detection reported by Wu *et al.* [43], the threshold in CHT algorithm is set to only detect outer ring of Airy disk below the focal plane, which has higher intensity value. The error of detection is estimated by the root-mean-square error between physical z -position, z , and fitted z -position, z_f , as $\sqrt{(z_f - z)^2} = 0.27 \mu\text{m}$.

2.4.4 Velocity calculation

Figure 2.9A and B show two consecutive sub-images, Frame $\mathcal{A}$ and $\mathcal{B}$, separated by time Δt . Figure 2.9C shows the same sub-image as Frame $\mathcal{A}$ and superimposed detected Airy disks from both sub-images (red contour from Frame $\mathcal{A}$ and blue contour from Frame $\mathcal{B}$). Note that not all features are identified as particles because in-focused particles are not used for z -position calculations. Here one can see that in many cases, a particle at t has a match at $t + \Delta t$. However, in some cases the one-to-one correspondence is less clear. This might be due to particles not being successfully identified in either the first or second frame, large particle displacement,

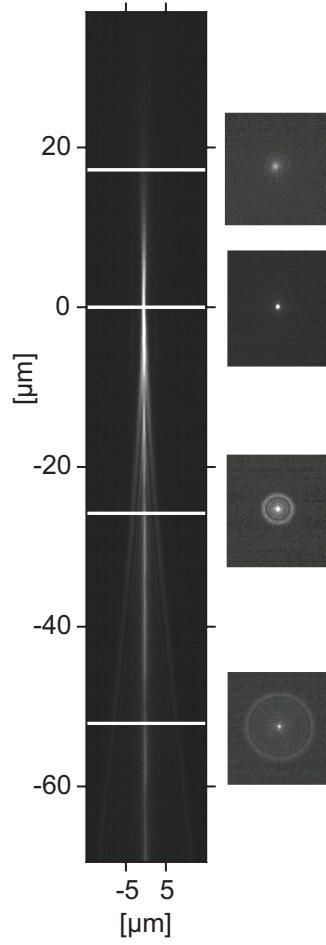


Figure 2.7: A light cone of a trapped fluorescent particle. 0 represents the z -position where the particle is in focus. Positive and negative value indicate above and below the focal plane respectively. The Airy disks in $x - y$ coordinate plane are shown by horizontal slices at four z -positions.

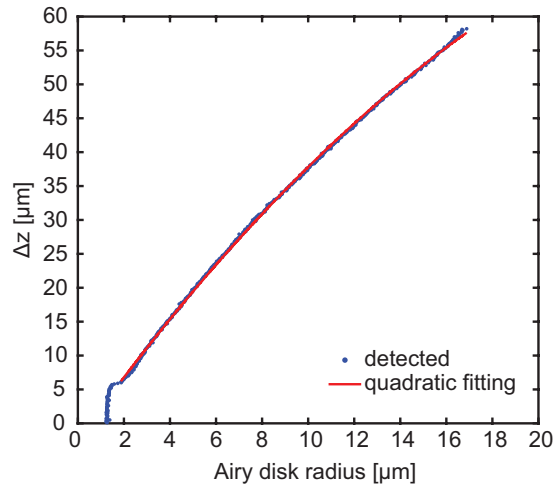


Figure 2.8: A reference library from detected Airy disk radius of an immobilized particle to its z -position, which is Δz distance below the focal plane. For the out-of-focus particle (radius larger than $2 \mu\text{m}$), the detected radii and z -positions follow a quadratic function.

high particle seeding or the presence of noise, all of which lead to confusion as how particles should be matched. For each particle, we define a spherical searching volume and identify all possible particle matches in three dimensions. The searching radius is an estimation of particle displacement, calculated based on the speed of active fluids [34] and the density of particle seeding. In each interrogation sphere, there are three possible outcomes:

1. No particle is found in the following frame, which means we lose track of a particle;
2. One particle, P'_1 , is detected solely in the interrogation sphere (Figure 2.9D) of the original particle, P_1 , in which case we link P_1 with P'_1 , announcing that P_1 moves to P'_1 after the time interval δt and calculate the displacement in the x , y and z axes respectively;
3. More than one particle is detected, as in the interrogation sphere of particle, P_5 (Figure 2.9D). In this case, we apply two conditions: that each particle cannot be linked more than once, and that each particle prefers to be linked to the one with the shortest separation, known as the “nearest neighborhood” method in particle tracking velocimetry [56]. The probability that an interrogation window yields a correct measurement by the nearest neighborhood method is given by the probability that the window contains exactly one matching pair of particle images. Following the probability analysis reported by Keane *et al.* [57] and Adrian *et al.* [58], we estimate that a particle concentration beyond a concentration 0.08% (v/v) in the sample will lead to a significant decrease of correct measurements with the nearest neighborhood method.

In this way, we are able to identify displacement of each particle from frame to frame, and via dividing by the frame rate, δt , we can define a particle velocity. Accuracy of

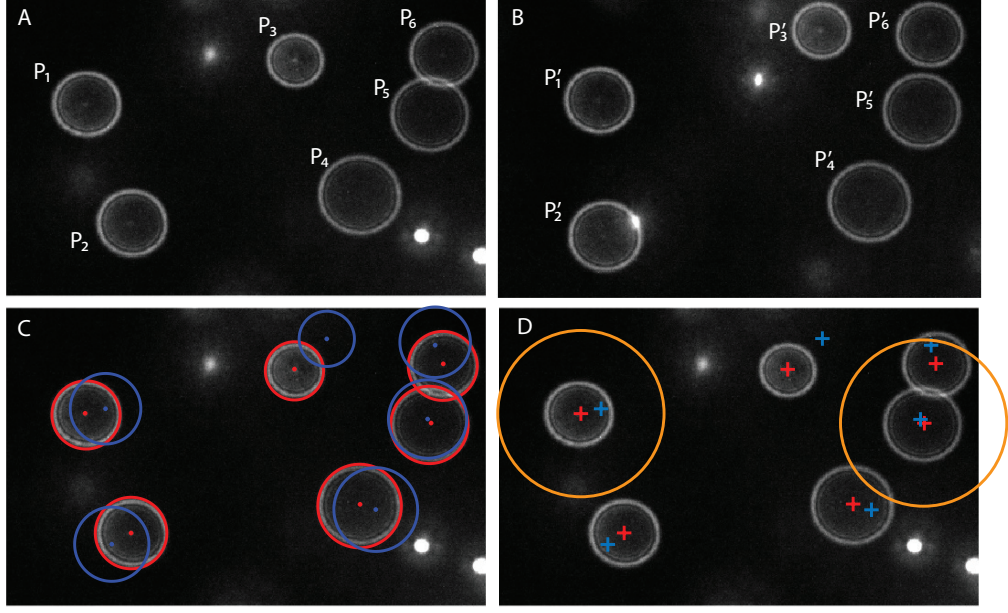


Figure 2.9: Displacement analysis of tracer particles by the nearest neighbor method. (A) Frame $\mathcal{A}$ recorded at time t . (B) Frame $\mathcal{B}$ recorded at time $t + \delta t$. (C) Particles detected in Frame $\mathcal{A}$ (red) and $\mathcal{B}$ (blue). Red and blue lines denote the outer contours of the Airy disks and dots denote the circular centers. (D) Determination of displacement by searching in an interrogation sphere (yellow) with a $7 \mu\text{m}$ radius. Each sphere centers at a detected particle in Frame $\mathcal{A}$ (red cross) and search for detected particles in Frame $\mathcal{B}$ (blue cross). Contrast of Frame $\mathcal{A}$ and $\mathcal{B}$ are enhanced for presentation purpose.

the three-dimensional particle tracking velocimetry is discussed in validation section of each chapter with specific settings of each experiment.

The piezo z-drive relocates the objective to multiple z -positions and enables a full observation of a three-dimensional volume by stacking images with respect to focal plane locations along z -axis. During the tracking period, a Lagrangian three-dimensional velocity field, as a function of tracking time and particle location, can be achieved by averaging over detected particles. In addition, an Eulerian three-dimensional velocity field can be achieved by averaging over particles at a fixed spatial coordinate. However, a delay period exists among different scanning positions. This “non-simultaneous” nature will be discussed in each chapter with respect to the specific speed of active fluids in various confining geometries.

CHAPTER THREE

Three-dimensional structure of coherent flows in kinesin-driven active fluids

3.1 Introduction

Active matter systems consist of active agents that are individually capable of converting free energy into systematic motion [5]. Active matter covers various types of biological materials, driven by internal active stress [59, 60], and can be extended to a broader context of self-propelled particles powered by external stress [5, 26]. Among the topics that have been investigated, confinement effect on active matter systems is emphasized because it provides insights into the fundamental principles of collective behavior and self-organization, across a wide range of length scales. Collective motion (such as cytoplasmic streaming or rotational flow) and self-assembled structures (like asters, spirals, vortices, etc.), introduced by boundary surfaces, have been investigated in cytoplasm [23, 61, 62], in bacterial suspensions [12, 39, 63], in cells [10, 11], in synthetic cytoskeletal extracts [35, 37, 64] and in self-propelled particles [14, 65]. Our attention focuses on specific cytoskeletal extracts, consisting of microtubules, kinesin clusters and depletion polymers. The depletion polymers induce depletion force to microtubule filaments through volume exclusion, which encourages bundling of microtubules. In antipolar microtubule pairs, the motor clusters convert chemical energy to sliding force, which gives rise to extensile flows in the system [20, 27]. The artificially assembled active system is referred as “active fluids” to indicate the predominate liquid-like property. Recent experiments elucidated two dynamical states of kinesin-driven active fluids, including a chaotic turbulent state and a long-ranged large-scale coherent state [28]. Transitions between these two states emerge in three-dimensional confinements, across micrometer to millimeter scale. In addition, the large-scale coherence is found to correlate with the nematic order extended from confining boundaries [28], similar to the “wetting” phenomenon in liquid crystals near boundary surfaces [66]. However, there are many questions remain unclear. For example, what is the structure in active flows? What

is the influence of confining surface condition? Besides, we do not understand why a chaotic-to-coherent transition appears around a certain aspect ratio across a wide range of confining geometries. To obtain three-dimensional measurements, we designed an experimental set-up that allows long-term imaging with fast scanning and reconstructed three-dimensional flow fields via tracking off-focus fluorescent tracer particles. In order to assess surface condition effect, we measured the dynamics and structure while altering materials and chemical treatments on confining boundaries.

3.2 Materials and methods

3.2.1 Channels

The active fluids were confined in a series of toroidal channels with equivalent inner radii, $r_{in} = 1$ mm (Figure 3.1). Dimensions of the tested channels are shown in Table 3.1. We define aspect ratio as the ratio between height, h , and width, w , therefore, h/w . Channels were manufactured either by etching silicon wafers (University Wafer, 444 and 452) or by CNC-machining cyclic olefin copolymer plates (TOPAS Advanced Polymers, 8007). Silicon wafers were etched using an inductively-coupled plasma reactive ion etching (RIE) system (SPTS Technologies, Model LPX-ICP), following the Bosch process [67] (Appendix A). The channel was sealed using a glass cover slide (Fisher scientific, 12-542C, 170 μm thickness). To ensure a consistent surface condition, the fabricated silicon channels and the cover slide were coated with polyacrylamide (Appendix B).

In order to assess the influence of different surface conditions, a toroid, with similar dimensions to one of the silicon channels (Si IV), was CNC-machined (MDA

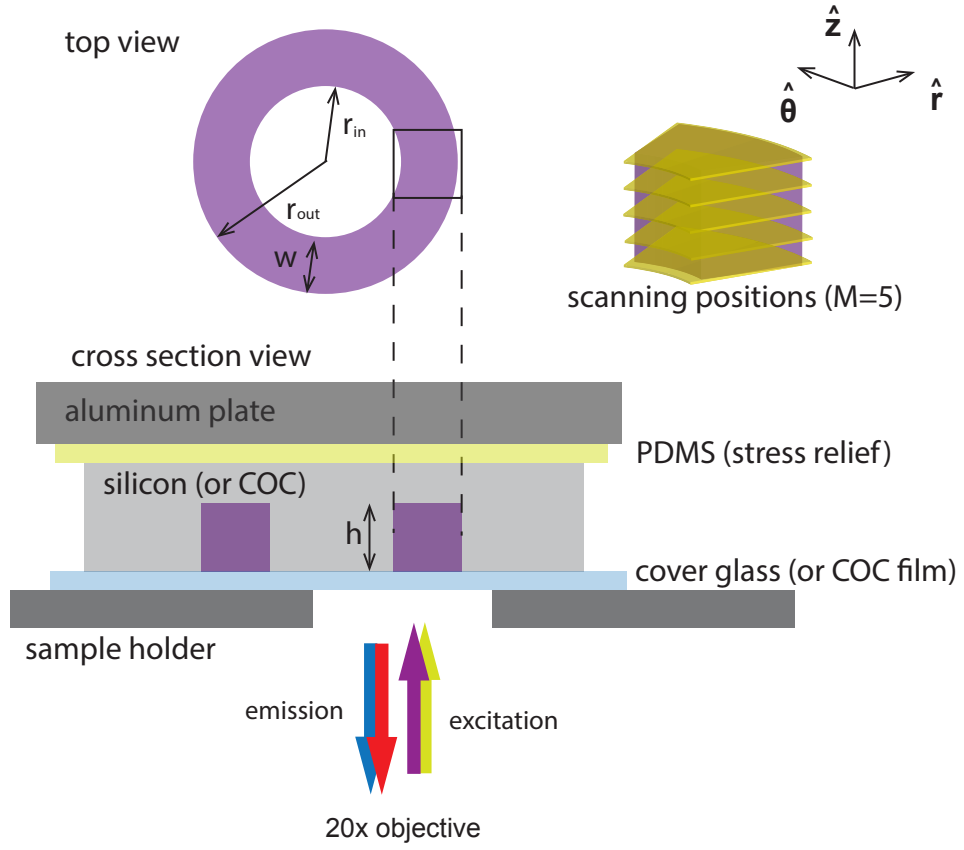


Figure 3.1: A two-dimensional sketch of an experiment with an epi-fluorescence microscope. Active fluids are sealed in a toroidal channel, packed in microfluidic devices, and scanned in multiple z -positions ($M = 5$ as an example). Scanning sections are highlighted planes in the three-dimensional outline. Active fluids are excited from below and emission light covers wavelengths of two colors.

Precision LLC., Model V8-TC8 3-axis) in a cyclic olefin copolymer (COC) plate (Appendix A). This channel was sealed using a COC film (TOPAS Advanced Polymers, 5013, $101.6 \mu\text{m}$ thickness, refractive index 1.5). The COC channel and cover film were coated by pluronic F-127 (Sigma, P2443) (Appendix B).

Table 3.1: Details about tested channels and experimental measurements.

material	abbreviation	w [μm]	h [μm]	h/w	coating	cover	M
silicon	Si I	325 ± 1.2	82 ± 0.9	0.25	polyacrylamide	glass	3
	Si II	111 ± 0.6	107 ± 0.7	0.96	polyacrylamide	glass	5
	Si III	218 ± 0.7	206 ± 0.3	0.94	polyacrylamide	glass	5
	Si IV	323 ± 1.2	306 ± 1.3	0.95	polyacrylamide	glass	10
cyclic olefin copolymer	COC	298 ± 2.2	292 ± 1.2	0.98	pluronic	COC film	10

3.2.2 Two-color active fluids

The active fluids measured here have been used extensively in previous studies [27, 34] and consist of a mixture of fluorescent microtubules, kinesin-streptavidin motor clusters, depletion polymers, an adenosine triphosphate (ATP) regeneration system and fluorescent spherical tracer particles (Appendix C). Microtubules were polymerized from tubulin dimers, which were labeled by Alexa Fluor 568 dye (Thermal Fisher Scientific, A20003, excitation/emission: 578 nm/603 nm) during the purification process. Motor clusters were obtained by mixing kinesin motors with streptavidin tetramers (Invitrogen, S-888). Pluronic micelles F127 (Sigma, P2443) in aqueous active systems encourage microtubule to form bundles by volume exclusion. The ATP regeneration system maintained a steady ATP concentration in assembled system to stabilize motion of kinesins (Appendix C). For active flow measurement, we dispersed $\sim 0.003\%$ (v/v) fluorescent particles with Fluo-Max Blue dye (1 μm diameter, Thermal Fisher Scientific, B0100, excitation/emission: 412 nm/447 nm) in the assembled kinesin-driven active fluids.

The active fluids were loaded into the channel and sealed by bolting together a microfluidic device (Figure 3.1) that included, from bottom to top, a sample holder which fit the motorized microscope stage (Prior Scientific, H101A), a treated cover (microscope cover glass or COC film), a treated channel (silicon channel or COC

channel), a polydimethylsiloxane (PDMS) layer, ~ 1 mm thick, to provide stress relief and an aluminum plate. The assembled package can prevent the sample from drying out for more than a week.

3.2.3 Two-color imaging and three-dimensional scanning

To build an experimental set-up with two-color imaging and three-dimensional scanning, following equipment were added to an epi-fluorescence microscope (Nikon TE 200 eclipse): a four-wavelength high power LED source (Thorlabs, DC4100), two-bandwidth optical components, an air immersion objective (Nikon CFI Plan Apo VC $20\times$, $WD = 1$ mm, $NA = 0.75$) held by a piezo z-drive (Physik Instrumente, Model P-725), an optical splitter (Cairn Research, Optosplit II) and an sCMOS camera (PCO edge 5.5). The microscope and associated equipment were contained inside an environmental chamber which maintained the temperature of the experiment at $25^{\circ}\text{C} \pm 0.3$.

Two LEDs (405 nm and 565 nm) of the LED light source were energized for illumination. The excitation light, refined using a two-bandwidth excitation filter (Chroma Technology, 59003x, 395/30 nm and 575/30 nm), was directed through the air immersion objective and a cover (microscope glass cover slide or COC film) to the active sample. In the sample, the fluorescently-labelled microtubules and fluorescent tracer particles emitted red and blue wavelengths respectively. The emission light passed through a two-bandwidth dichroic mirror (Chroma Technology, 59003bs, transmission in 460/30 nm and 630/60 nm) and was directed to an optical splitter that separated the two colors using a dichroic mirror (Chroma Technology, 565dcxr). The light streams passed through band-pass filters (Chroma Technology, ET450/50m and D620/60m respectively) in the optical splitter before being projected onto two

halves of the sCMOS sensor. Although the full sensor size of the camera is $2560 \text{ pixels} \times 2160 \text{ pixels}$, limitations with the windows of the optical splitter resulted in a final image of $2500 \text{ pixels} \times 1300 \text{ pixels}$. The exposure time was 25 milliseconds. The camera settings and image recording were controlled using CamWare64 software (PCO). A recording frequency of 1 Hz was used for Airy disk calibration, Brownian motion validation, and measurement of chaotic active flows in the toroidal channel. A higher frequency, 5 Hz, was used to record coherent active flows in the toroidal channels.

The three-dimensional scanning was accomplished by the piezo z-drive, which can hold the objective and scan up to $400 \text{ }\mu\text{m}$ along the z -axis. The z-drive accepts a voltage input between $[0, 10] \text{ V}$, where 0 V represents the home position and 10 V represents the maximum position ($400 \text{ }\mu\text{m}$ above); other positions are linearly related to the input voltage. During experiments, the scanning positions were provided by a controlling computer, using a digital-to-analog converter (National Instruments, DAQ PCI6052e). For the purpose of synchronization (Figure 3.2), the LED and the camera were triggered by the rising edge of a timing pulse (Berkeley Nucleonics Corp., Model 577), while the digital-to-analog converter was triggered by the falling edge. This timing ensured that the excitation and recording took place while the objective was stationary. The number of scanning positions, M , along the z -axis is limited by the imaging frequency (maximum: 5 Hz), the height of confining geometry (maximum: $\sim 300 \text{ }\mu\text{m}$) and the settling time of the piezo z-drive (maximum: 100 milliseconds).

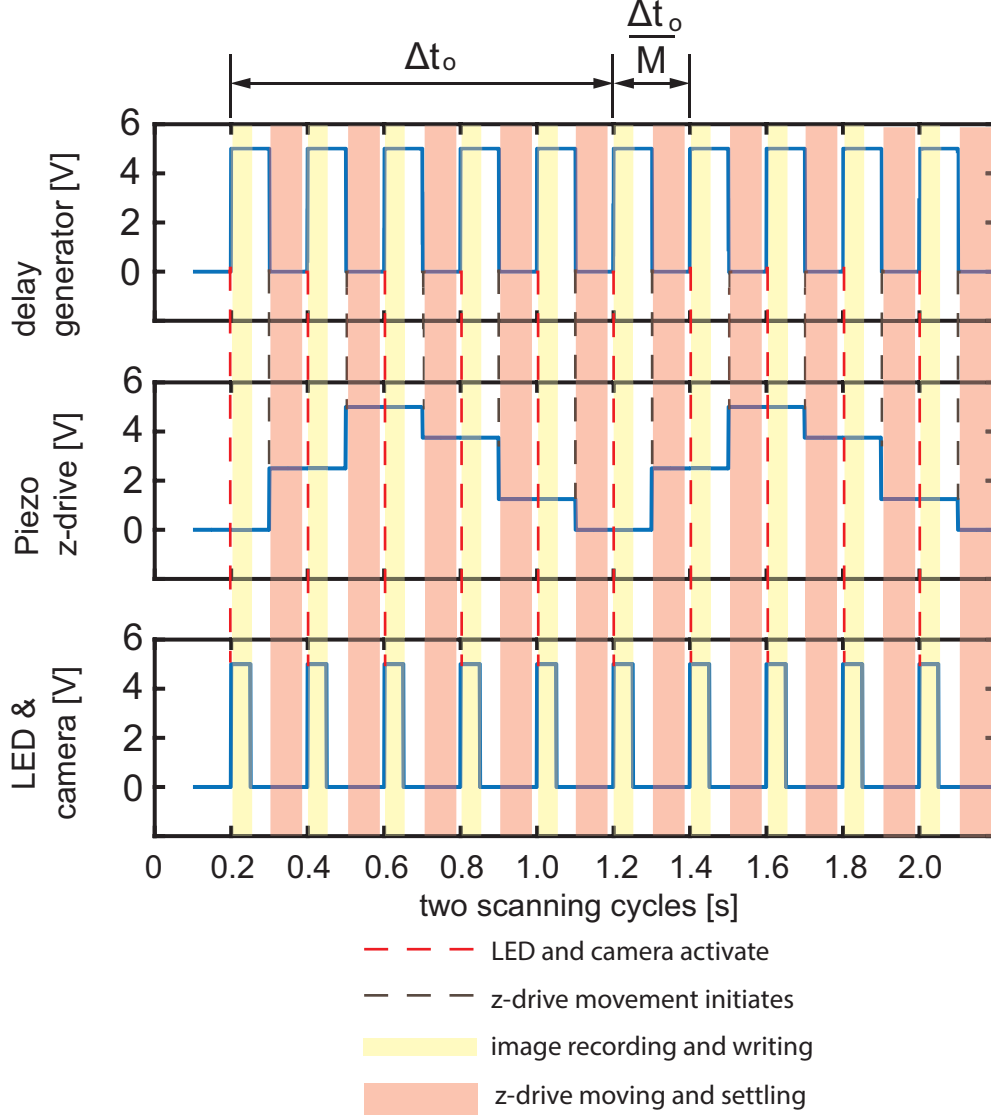


Figure 3.2: Signals of synchronized equipment during two consecutive scanning cycles. Top: a timing pulse from the digital delay generator. Middle: a step function that triggers the piezo z-drive to cycle in several z -positions. In this case, the step function is programmed for a 200 μm range of scanning with five z -positions. Bottom: an external trigger for illumination and image recording. Rising edges of the timing pulse (red dash line) activate the LED and the camera while falling edges of the timing pulse (brown dash line) trigger the movement of z-drive. Images are recorded and written during yellow bands. The piezo z-drive moves and settles during orange bands.

3.2.4 Three-dimensional particle tracking velocimetry

The three-dimensional positions were acquired by detecting the Airy disks associated with out-of-focus particles [42, 43, 44, 51]. The Airy disk radius reflects the z -position of an out-of-focus particle, and the Airy disk center determines the particle’s location in the horizontal plane. Therefore, three-dimensional particle tracking is transformed into a circle detection problem.

The position and size of the Airy disks were detected using a well-developed method, the Circle Hough Transform (CHT) [52]. The detection code, written in MATLAB, followed established pattern recognition concepts [53], the CHT algorithm [45] and parallel processing methods (MATLAB Imaging Processing Toolbox). A reference library, which correlates the Airy disk radius with the z -location of a particle, was created by imaging a $1\text{ }\mu\text{m}$ diameter fluorescent particle, immobilized in 1% (w/w) agarose gel, over a range of $100\text{ }\mu\text{m}$ along z -axis in $0.1\text{ }\mu\text{m}$ increments. Scanned images were stacked together to form a light cone (Figure 3.3) [51].

In each frame of an experimental sequence, the three-dimensional position of detected particle was determined, as described above. After repeating the detection for every frame, a time history of each particle’s position was formed. The displacement of a given particle, between a pair of successive frames, was estimated using the “nearest neighborhood” method [56]. The velocity was calculated using a first-order finite difference.

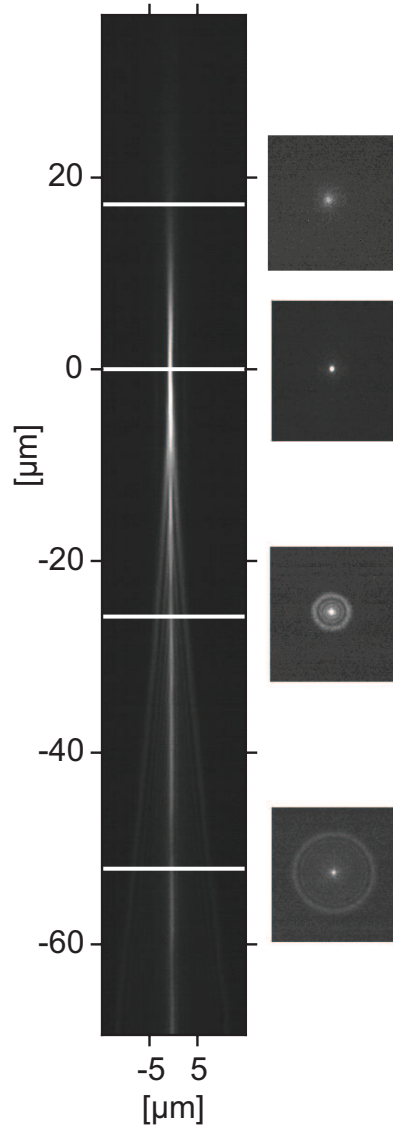


Figure 3.3: A light cone of a trapped fluorescent particle. 0 represents the z -position where the particle is in focus. Positive and negative value indicate above and below the focal plane respectively. Airy disks are shown by horizontal slices at four z -positions.

3.2.5 Data collection and analysis

Before the data collection of confined active fluids, we performed a validation experiment for the imaging system and the three-dimensional particle tracking. Details about the accuracy of three-dimensional measurements are discussed in Appendix D. The data acquisition was repeated twice in the same condition from two-color active fluids confined in toroidal channels. The scanning and image acquisition were initiated within 200 seconds since the active fluids were assembled. A portion of the entire toroidal channel, around a 20° slice, was observed at multiple z -positions (Figure 3.4).

In this chapter, we focus on the data analysis associated with the motion of the passive tracer particles. Results from fluorescent microtubules will be addressed separately.

At each scanning position, the image sequence consists of N images, taken at a frequency, $f = 1/\Delta t_o$, where Δt_o is the temporal resolution, typically 1 second for $M = 5$ (Figure 3.2) and 2 seconds for $M = 10$. In a complete data set, image sequence recorded at the final z -position in a full scanning cycle is delayed, compared with the initial z -position, for a time period of $(M - 1) \cdot \Delta t_o / M$. Due to the low-speed movements of active materials, typically around $2 \mu\text{m/s}$ in the concentration of the active fluids we studied here [28, 34], particles move less than $4 \mu\text{m}$ during the delayed period. Therefore, sequences from all scanning positions are treated as “simultaneous” parallel recordings. Results are averaged over M sequences from all scanning positions and over two repeated experiments in each confining geometry.

Each image at a static position, corresponding to time $t_k = k\Delta t_o$ ($k = 1, 2, \dots, N$), has P_k particles, and each particle in that image, denoted by the index j ($j =$

$1, 2, \dots, P_k$), has a position, $\mathbf{R}^j$ (in a cylindrical coordinate system):

$$\mathbf{R}^j(t_k) = (r^j(t_k), \theta^j(t_k), z^j(t_k)). \quad (3.1)$$

Particles are detected by CHT from frame to frame in the sequence, and from this time history of particle positions, the velocity vector of the individual particle is defined as

$$\mathbf{u}^j(t_k) = (u_r^j(t_k), u_\theta^j(t_k), u_z^j(t_k)), \quad (3.2)$$

where each component is derived using a backward Euler method

$$\begin{aligned} u_r^j(t_k) &= \frac{r(t_{k+1}) - r(t_k)}{\Delta t_o}, \\ u_\theta^j(t_k) &= r(t_k) \cdot \frac{\theta(t_{k+1}) - \theta(t_k)}{\Delta t_o}, \\ u_z^j(t_k) &= \frac{z(t_{k+1}) - z(t_k)}{\Delta t_o}. \end{aligned} \quad (3.3)$$

During a time period, $T = [t_s, t_e]$, when dynamics are relatively stable, we average over the number of particles, P_k , in the image at each time, t_k , and over the period T , covering N recorded images, where

$$t_e = t_s + N\Delta t_o. \quad (3.4)$$

For all experiments discussed here, the statistics are calculated from the same period, T , from $t_s = 1$ second to $t_e = 2000$ seconds ($k = t_s \cdot f, (t_s + 1) \cdot f, \dots, t_e \cdot f$).

Following the standard turbulence literature (for example [68]), we employ the “Reynolds decomposition” for the velocity:

$$u_i(t_k) = \bar{u}_i + u'_i(t_k) \quad (i = r, \theta, z), \quad (3.5)$$

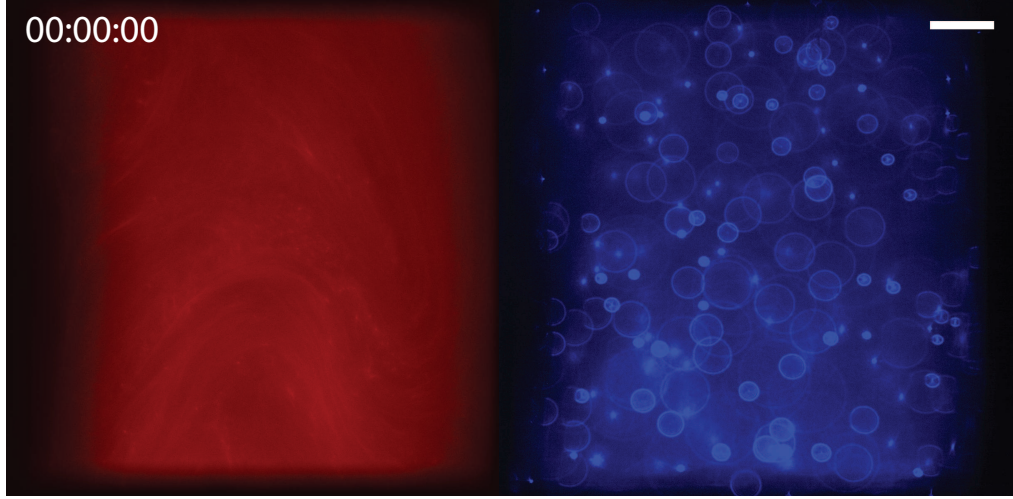


Figure 3.4: An image frame of two-color active fluids in a silicon channel (Si IV) at a scanning level, z , where $z = 100 \mu\text{m}$. Red window shows microtubule bundles and blue window shows tracer particles. Scale bar: $50 \mu\text{m}$.

where the average velocity, $\bar{u}_i$, denotes a temporal average over $2M \cdot N$ images, collected from all scanning positions, M , among two repeated experiments during the period $T = [t_s, t_e]$:

$$\bar{u}_i = \frac{1}{2M \cdot N} \sum_{t_s}^{t_e} \frac{1}{P_k} \sum_{j=1}^{P_k} u_i^j(t_k). \quad (3.6)$$

The variance of the fluctuating velocity, $\overline{u_i'^2}$, is defined as:

$$\overline{u_i'^2} = \sigma_i^2 = \frac{1}{2M \cdot N} \sum_{t_s}^{t_e} \frac{1}{P_k} \sum_{j=1}^{P_k} (u_i^j(t_k) - \bar{u}_i)^2. \quad (3.7)$$

Among different confinements, our observation volume is restricted in $\Delta\theta$ along azimuthal direction, where $\Delta\theta \approx 20^\circ$. Since the coherent flows are statistically homogeneous in the θ -direction, the observation volume can be averaged along the azimuthal direction, therefore, velocity profiles can be projected along two axes: r -axis (“top view”) and z -axis (“side view”). From a practical perspective, velocity vectors are binned to describe the flow so that vectors from a small range of separation

are averaged together. In the “top view”, we present mean azimuthal velocity as a function of its radial distance with respect to the inner wall of a channel, $\Delta r = r - r_{in}$, thus:

$$\langle u_\theta(\Delta r) \rangle \cong \frac{1}{P_r} \sum_{j=1}^{P_r} u_\theta^j(\Delta r - \delta_r < \Delta r < \Delta r + \delta_r). \quad (3.8)$$

Here, the angle brackets, $\langle \dots \rangle$, denote an ensemble average over particles, P_r , collected within the separation distance, $2\delta_r$, around Δr from repeated experiments. δr varies between $7 \mu\text{m}$ to $20 \mu\text{m}$ in different dimensions. The maximum of the radial distance is the corresponding width of each channel, $\text{Max}(\Delta r) = w$. In the “side view”, mean azimuthal velocity is demonstrated, in the same manner, as a function of its vertical position, z , therefore:

$$\langle u_\theta(z) \rangle \cong \frac{1}{P_z} \sum_{j=1}^{P_z} u_\theta^j(z - \delta_z < z < z + \delta_z), \quad (3.9)$$

where P_z is the particle number collected within the small separation distance, $2\delta_z$, around z from repeated experiments. δz varies between $7 \mu\text{m}$ to $20 \mu\text{m}$ in different dimensions. $z = 0$ refers to the surface of the glass cover slide or the COC film so that the maximum is the corresponding height of each channel, $\text{Max}(z) = h$.

3.3 Results

3.3.1 Active flows: a chaotic turbulent state and a coherent state

Figure 3.5 illustrates the three components of velocity as a function of time from three typical experiments, selected from 10 data sets in total recorded from 5 different channels. The radial and vertical velocity components always fluctuate around a zero mean. The existence of a coherent flow is defined by the presence of a non-zero azimuthal velocity. Figure 3.5A shows chaotic flows in the silicon channel (Si I) with a small aspect ratio, $h/w = 0.25$. The azimuthal velocity fluctuates around zero. Figure 3.5B indicates the presence of a coherent flow on top of the chaotic turbulent state in the silicon channel (Si IV) with a large aspect ratio, $h/w = 0.95$. The azimuthal component starts at around $2 \mu\text{m/s}$ and decays monotonically towards $0.5 \mu\text{m/s}$, indicating the existence of long-term coherence for over 9 hours. Azimuthal velocity components, measured from other silicon channels (Si II and Si III), present similar decays as a function of time with smaller initial speeds (results not shown here). Figure 3.5C shows the velocity components measured in the COC channel. As with the silicon channels, the x - and z -components of velocity fluctuate about zero. The θ -component starts off with a non-zero mean, and decays as the experiment proceeds. However, unlike the cases of silicon channels, the lifetime of the active flow in the COC channel is much shorter, lasting approximately 3 hours. The yellow-highlighted areas in the three plots define a time window of $T = 2000$ seconds over which the temporal averaging of velocity was performed for the subsequent data analysis.

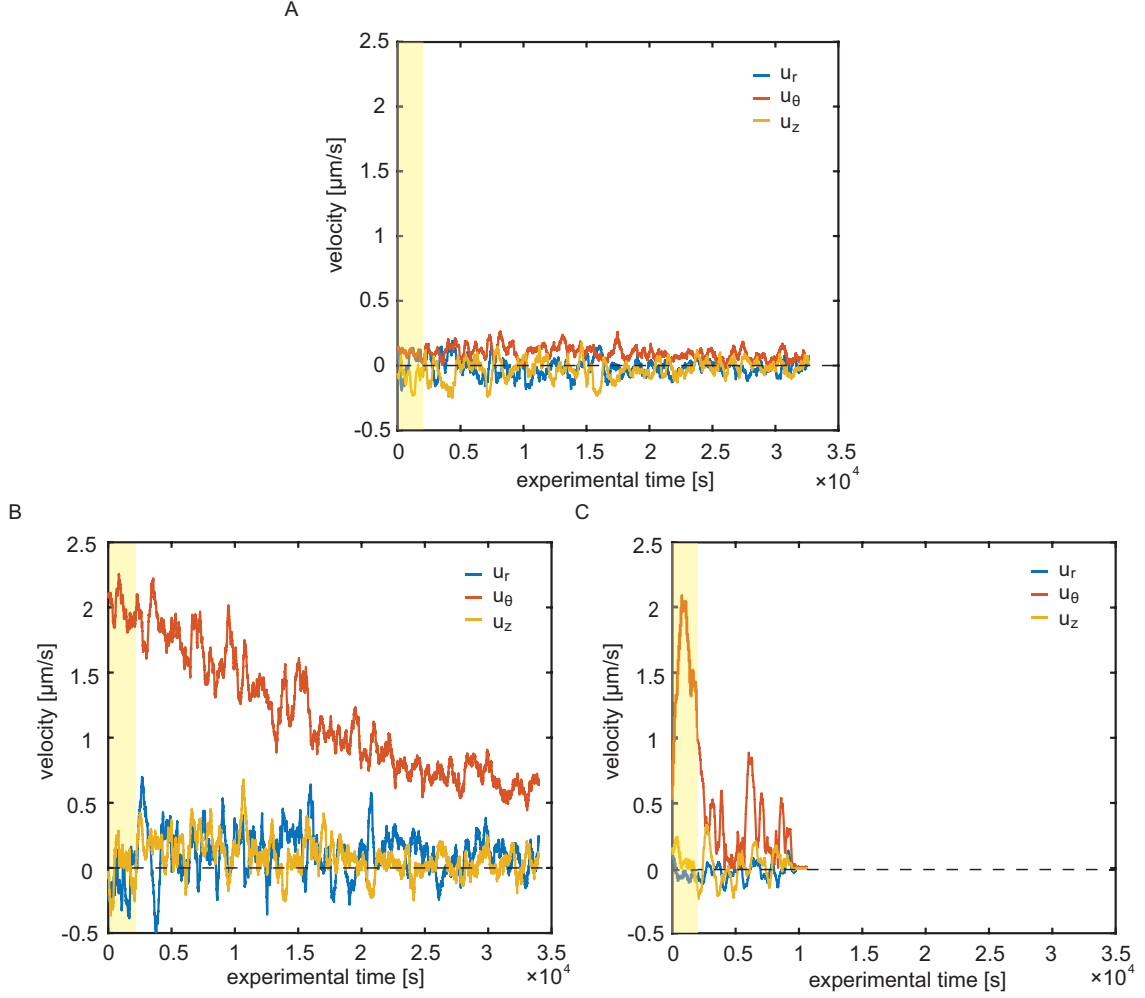


Figure 3.5: (A) Velocity of chaotic turbulent flows in the silicon channel (Si I) as a function of experimental time. (B) Velocity of coherent flows in the silicon channel (Si IV) as a function of experimental time. (C) Velocity of coherent flows in the COC channel. All velocity values are locally smoothed over a neighbor period of 200 seconds. The highlighted area defines a time period, $T = [t_s, t_e]$, over which temporal averaging takes place.

3.3.2 Velocity of active flows

Figure 3.6A shows the averaged velocities (Equation 3.6) from silicon channels (filled circles) and the COC channel (empty circles) over the defined time period, T . At the smallest aspect ratio (Si I), the average velocities are all zero, while at the large aspect ratios (Si II, III, IV and COC), the average azimuthal velocity rises with the channel height. Figure 3.6B shows the velocity fluctuations (Equation 3.7) measured in both the silicon channels (filled squares) and the COC channel (empty squares), as a function of the channel depth over the same time period. Fluctuations in two silicon channels with smaller heights (Si I and Si II) lie around the same magnitude, $1 \mu\text{m/s}$, though they are in completely different dynamical states. For geometries with coherent circulations (Si II, Si III, Si IV and COC), all three components of fluctuations increase as channel size expands.

3.3.3 Three-dimensional structure of active flows

The azimuthal velocities, averaged in the z and θ directions (Figure 3.7A) or in the r and θ directions (Figure 3.7B), indicate a well-defined symmetric velocity structure present in the active systems. In the shallowest channel (Si I), consistent with previous results (Figure 3.6A), there is no mean velocity structure. However, in the deeper channels, velocity profiles of coherent flows vary from exhibiting a single peak (Si II and Si III) to four distinct peaks (Si IV) centered near the four corners of the channel (around $50 \mu\text{m}$ to $60 \mu\text{m}$ away from boundaries).

Figure 3.8A and B compare the velocity profiles from coherent flows in the largest silicon channel, Si IV, and the comparably-sized COC channel. The peak separation in the Si IV channel is not observed in the COC channel, which indicates a single

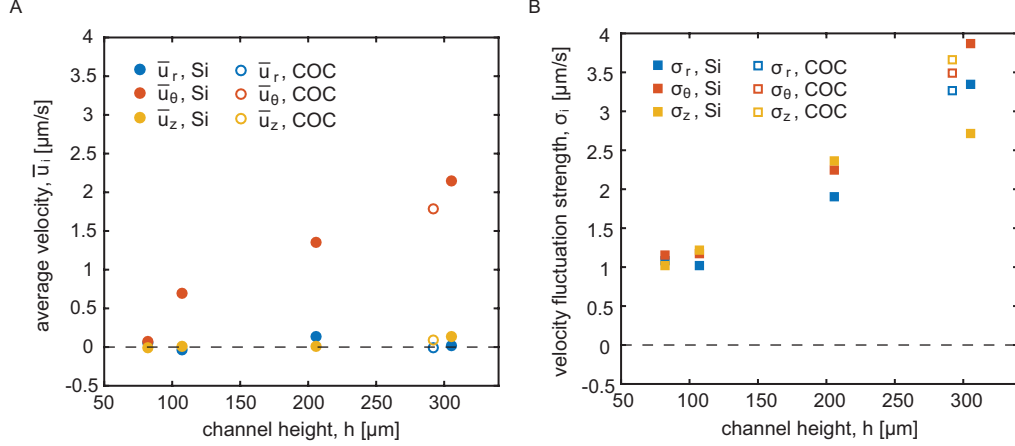


Figure 3.6: (A) Average velocity of the active fluids in multiple channels, averaged during the defined time period, T . Filled circles are data from silicon channels and empty circles are data from the COC channel. (B) Velocity fluctuation strength of the active fluids in multiple channels during the time period, T . Filled squares are data from silicon channels and empty squares are data from the COC channel.

“plug”-flow similar to the profiles observed in the smaller silicon channels (Figure 3.7). In all cases, a “no-slip” boundary condition is apparent, although the spatial averaging near the walls may obscure some surface motion close to the confining boundary.

3.4 Discussion

3.4.1 The aspect ratio and the flow structure

Our observations are consistent with previous study [28], which illustrates a chaotic-to-coherent transition with an aspect ratio, h/w , around 0.4. In deeper channels (Si II, III, IV and COC), the large-scale coherence and the mean flow structure persist. We present “Poiseuille-like” profiles with a maximum around $1 \mu\text{m/s}$ (Si II in Figure 3.7A), and around $1.6 \mu\text{m/s}$ (Si III in Figure 3.7A), comparable to similar size COC toroids measured by Wu *et al.* [28]. In the shallowest channel (Si I), the

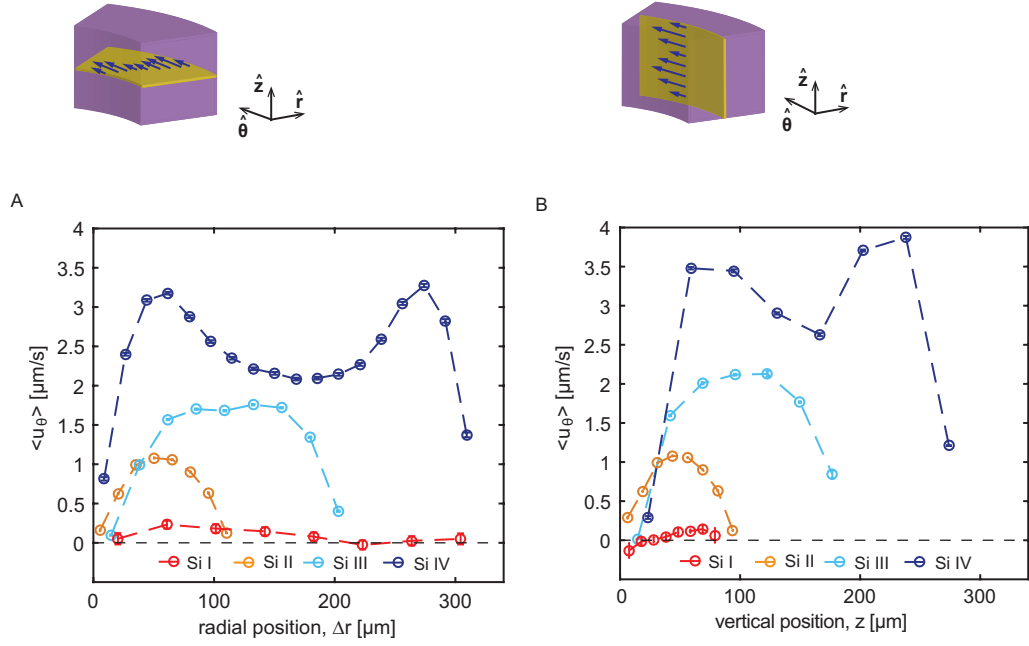


Figure 3.7: (A) Mean azimuthal velocity along r -axis from silicon channels. Radial position is defined as $\Delta r = r - r_{in}$, where r_{in} is the inner radii of toroidal channels, typically $r_{in} = 1$ mm. (B) Mean azimuthal velocity along z -axis from silicon channels. Vertical position is presented as z , with respect to the position of cover surface.

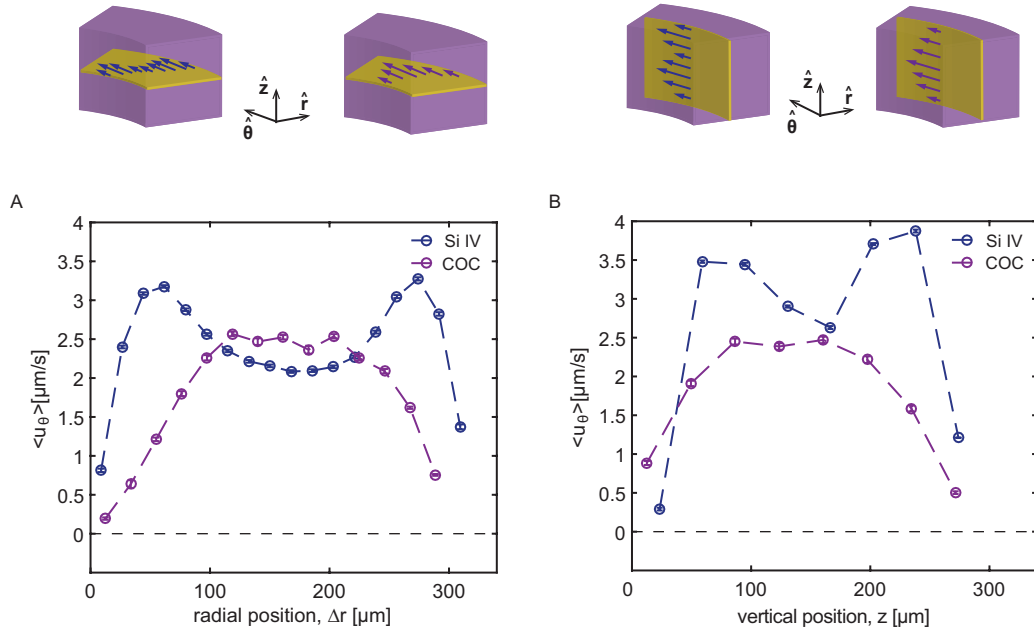


Figure 3.8: (A) Mean azimuthal velocity along r -axis from the silicon channel (Si IV) and the COC channel with altered surface conditions. Radial position is defined as $\Delta r = r - r_{in}$, where r_{in} is the inner radii of both channels, typically $r_{in} = 1$ mm. (B) Mean azimuthal velocity along z -axis from a silicon channel and a COC channel with altered surface conditions. Vertical position is presented as z , with respect to the position of cover surface.

aspect ratio does not satisfy the chaotic-to-coherent transition, and the mean flow structure vanishes. Therefore, the aspect ratio determines the structure of active flows. However, no previous measurement reported the peak separation (Figure 3.7, Si IV), which is caused by surface condition alternation.

3.4.2 Surface condition

Surface condition affects the structure of coherent flows. Figure 3.8 suggests that the polyacrylamide-coated silicon surface has a shorter effective length on the flow structure, compared with the pluronic-coated COC surface. Previous experiments by Wu *et al.* measured the average anchor angle of microtubule bundles, which is $\sim 20^\circ$ near the pluronic-coated COC surface [28]. Therefore, the anchor angle of microtubule bundles is estimated to be smaller than $\sim 20^\circ$ near the polyacrylamide-coated silicon surface. We predict that in a silicon channel, with the same surface treatment and a large enough width, active flows can be chaotic far away from boundaries.

Surface condition also influences the lifetime of coherent flows. We propose two hypotheses for lifetime variation. (i) The pluronic micelles, which form the coating on the hydrophobic COC surface, cause a dynamical variation of microtubule bundles in active flows. Previous experimental results by Henkin *et al.* show that a concentration increase of a different depletion polymer, polyethylene glycol (PEG), leads to a quick slow down in the microtubule bundles speed [34]. In addition, the leak of pluronic micelles from the coated boundary expose the hydrophobic COC surface, which further inhibits the flow of microtubule bundles. The exposure of hydrophobic COC boundary can also explain the sharp decline of the azimuthal velocity in Figure 3.5C. (ii) Kinesin clusters may be accumulating on the pluronic coating of the COC

surface. At molecular scale, a kinesin motor only hydrolyzes ATP when it binds to and walks along a microtubule filament [32]. Consequently, the ATP consumption rate is solely dependent on the concentration of kinesin motors and the period that motors stay binding to microtubules [32, 69]. When kinesin clusters are accumulating on the boundaries, less and less motors are effectively walking on microtubules and the chemical energy in the active fluids is not completely exhausted.

In general, surface condition alternation affects the duration and structure of coherent flows but does not appear to influence the circulation speed and fluctuation amplitude in initial period of coherent flows. The coherent speed is dependent on the dimension of channel cross section while the fluctuation amplitude is solely dependent on the smallest dimension, which is the height h , of the channel geometry.

3.4.3 Scaling of coherent flows

To normalize coherent flows by variations of velocity fluctuations (which are dependent on confinement geometries), we scale average azimuthal velocity (Equation 3.6) by velocity fluctuation strength (Equation 3.7), written as:

$$\tilde{u}_\theta = \frac{\bar{u}_\theta}{\sigma_\theta}. \quad (3.10)$$

Figure 3.9 presents scaled azimuthal velocity, $\tilde{u}_\theta$, in silicon channels (filled bars) and the COC channel (open bar). For the turbulent case, scaled θ -velocity shows a low level (less than 0.07) and for coherent cases, scaled θ -velocities from different channels remain in a high level, between 0.5 to 0.6. Therefore, as the aspect ratio is determined, coherent flows during peak time period scale with the fluctuation strength, σ_θ , regardless of geometrical dimensions or surface conditions. With the

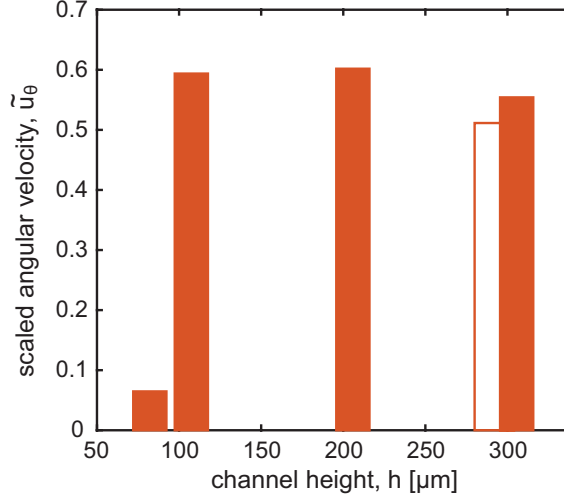


Figure 3.9: Scaled azimuthal velocity, $\tilde{u}_\theta$, as a function of channel height, h , from silicon channels and the COC channel. The lowest bar shows scaled azimuthal velocity of chaotic flows in a silicon channel (Si I) with a small aspect ratio. Others are obtained from coherent flows in various geometries with similar aspect ratios. Filled bars represent silicon channels and the empty bar represents the COC channel.

same concept, we normalize velocity profiles with dimension, as $\langle u_\theta(\Delta r/w) \rangle / \sigma_\theta$ and $\langle u_\theta(z/h) \rangle / \sigma_\theta$, where w and h are channel width and channel height in Table 3.1. The peaks of velocity profiles (Figure 3.10A and B) collapse to the same level, which again suggests that coherent flows from different geometries can be scaled.

3.4.4 Large-scale coherence

The coherent flows have remarkably large scales, compared to the average length of microtubule filament (1 μm [28]). In other active materials, similar large-scale coherence has been reported as “cytoplasmic streaming” or “rotational flows”. Cytoplasmic streaming [23, 61, 62], similar to active fluids discussed here, is generated by molecular motors walking on cytoskeletal filaments in the presence of organelle bound in living cells. Due to the restrictions from organelle or cell boundary, directed flow of cytosol shows a characteristic length scale around tens to a hundred micrometers. The self-organized rotating flows in bacterial suspensions, caused by

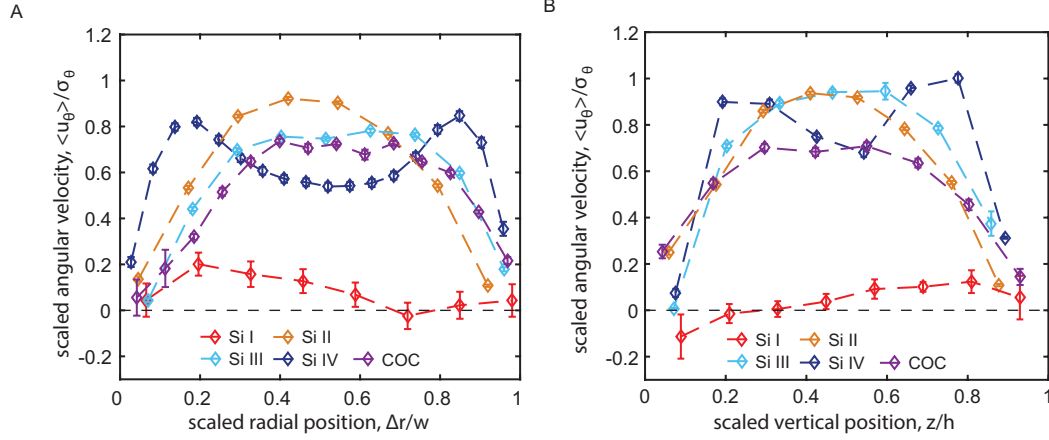


Figure 3.10: (A) Scaled velocity profile as a function of radial position. The radial position is normalized by corresponding channel width. (B) Scaled mean azimuthal velocity profile as a function of vertical position. The vertical position is normalized by corresponding channel height.

geometrical asymmetry or dimension variation in confinements, show characteristic length scale around tens to a hundred micrometers as well [12, 39, 63, 70]. In other artificially purified cytoskeletal extracts, wall-induced rotational flows emerge in a wide range of confining geometries, from tens to several hundred micrometers [35, 37, 64]. Though our observation and above reports share very similar circulating pattern, a major difference exists. Coherent flows in confined kinesin-driven active fluids are 3D phenomena that can not reduce dimensionality, whereas, above reports are quasi-2D phenomena.

3.5 Conclusion

We measured three-dimensional turbulent and coherent dynamics in the active fluids. Both lifetime and structure of coherent flows are affected by the surface condition on confining boundaries. Coherent flows show longer lifetime and multiple peaks when confined by silicon surfaces with polyacrylamide coating. The hypotheses about the reason of lifetime variation require further experimental investigations. One in-

teresting proposal would be varying the concentration of pluronic for the surface treatment of the COC channel. Another interesting proposal would be to repeat the same measurements with fluorescently-labelled kinesin-streptavidin clusters and fluorescent particles, which will reveal the motion of motor clusters and the dynamics of the active flows. In addition, we find that coherent flows can be scaled by fluctuations across different geometries and surface conditions. It encourages more exploration on the physical meaning of fluctuation in the active flows.

CHAPTER FOUR

**The loss of isotropy due to
confinement in kinesin-driven
active fluids**

4.1 Introduction

Molecular motors and cytoskeletal networks in living cells cooperate continuously and precisely to accomplish mechanical tasks in bounded environment, such as replication and division [71, 72]. Confinement imposes asymmetry to active stress, generated by thousands of nano-sized motors, and affects dynamics of cytoskeleton [73, 74]. Self-organizing patterns (vortex-like structures and spirals) and long-ranged collective motion, formed in artificially purified cytoskeletal extracts with spatial confinements, rise from the asymmetry and scale from tens of micrometers to a few millimeters [28, 35, 64, 75, 76]. Understanding the influence of confinement on synthetic active materials provides insight into the fundamental principles of self-organization and collective motions. We focus on a specific biological system, assembled from microtubule filaments, kinesin-streptavidin clusters and depletion polymers. Microtubule filaments form bundles in the presence of pluronic micelles. The cooperation of motor clusters converts chemical energy to sliding motion between antipolar microtubules, which gives rise to extensile flows in the system [27]. Recent experiments elucidated two dynamical states of the active fluids [28]: a chaotic turbulent state and a coherent state, which emerged in confined geometries across micron scale to millimeter scale. The emergence of the large-scale coherent circulation demonstrates that a scale-invariant criterion of three-dimensional confinement can effectively complete the transition between two dynamical states [28]. Our previous experiments (Chapter 3) profile the coherent flows in three dimensions and prove that the circulation scales with fluctuation in active flows. However, more questions arise from here, such as the physical meaning of fluctuation in flows, the correlation between fluctuation and confining geometry, and the response of isotropic active fluids to confining boundaries.

4.2 Materials and methods

4.2.1 Channels

Experiments were conducted in a series of cuboid channels with various heights, H , along z -axis (Figure 4.1). Dimensions of the tested channels are shown in Table 4.1. Except for the largest 4 mm cube, width, W , and length, L , of the channels remain the same. The channels were designed and fabricated using CNC-machining (MDA Precision LLC., Model V8-TC8 3-axis) in cyclic olefin copolymer (COC) plates (TOPAS Advanced Polymers, 5013L-10 and 8007). Details about channel fabrication are discussed in Appendix A. The channel was sealed using a glass cover slide (Fisher scientific, 12-542C). To ensure a consistent surface condition, the fabricated COC channels and the cover slide were coated with polyacrylamide (Appendix B).

Table 4.1: Experimental details in different channel geometries.

channel dimension			objective		focal plane	particle	tracking
W [mm]	L [mm]	H [mm]	WD [mm]	NA	Δz [mm]	diameter [μm]	dimensions
4.0	4.0	4.0	6.9	0.45	2.00 *	2.9	2D
2.0	2.0	2.0	1.0	0.75	0.80 **	1.0	3D
2.0	2.0	1.5	1.0	0.75	0.75	1.0	3D
2.0	2.0	1.0	1.0	0.75	0.50	1.0	3D
2.0	2.0	0.5	1.0	0.75	0.25	1.0	3D
2.0	2.0	0.3	1.0	0.75	0.15	1.0	3D
2.0	2.0	0.1	1.0	0.75	0.05	1.0	3D

* Using the extra long working distance objective.

** Focal plane position is restricted by the objective working distance.

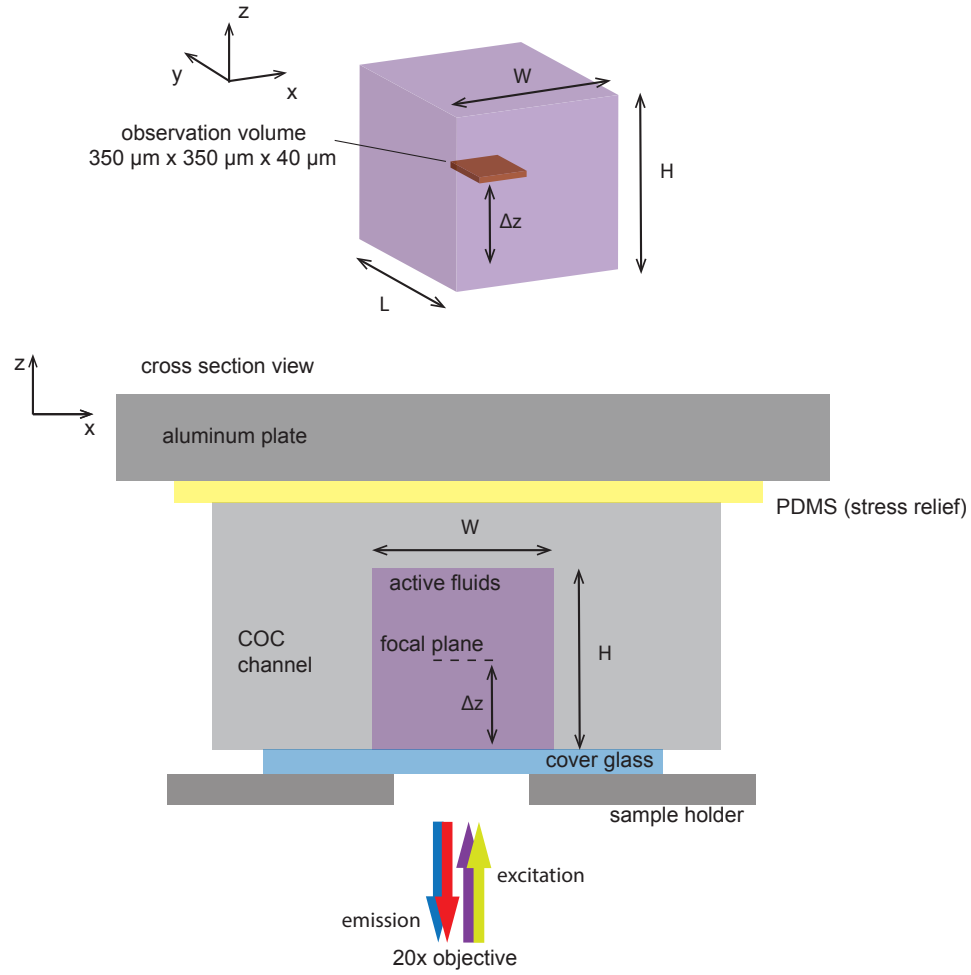


Figure 4.1: A two-dimensional sketch of an experiment with an epi-fluorescence microscope. Active fluids are sealed in a cubic channel and packed in microfluidic devices. Experimental observation volume is illustrated in the three-dimensional outline of the channel. Active fluids are excited from below and emission light covers wavelengths of two colors.

4.2.2 Two-color active fluids

The active fluids measured here were assembled from fluorescent microtubules, kinesin-streptavidin motor clusters, depletion polymers, an adenosine triphosphate (ATP) regeneration system and fluorescent spherical tracer particles (Appendix C). Microtubules were polymerized from tubulin dimers, labeled by Alexa Fluor 568 dye (Thermal Fisher Scientific, A20003, excitation/emission: 578 nm/603 nm) during purification process. Motor clusters were obtained by mixing kinesin motors with streptavidin tetramers (Invitrogen, S-888). Pluronic F127 (Sigma, P2443) formed micelles in aqueous systems and encouraged microtubule filaments to bundle. The ATP regeneration system maintained a steady ATP concentration in the active fluids to stabilize the motion of kinesins. For active flow measurement, we dispersed $\sim 0.001\%$ (v/v) fluorescent particles in the aqueous samples. As shown in Table 4.1, fluorescent particles, Purple Plum ($2.9\ \mu\text{m}$ diameter, excitation/emission: 395 nm/428 nm, Bangs Laboratories, FS05F), were used in the largest cubic channel, $H = 4\ \text{mm}$. Different fluorescent particles, Fluoro-Max Blue ($1\ \mu\text{m}$ diameter, excitation/emission: 412 nm/447 nm, Thermal Fisher Scientific, B0100), were used in other geometries. The alternation between particles was aimed at selecting appropriate size of spheres for particle tracking in different channels. Both types of particles have close spectra so that they can share the same set of optical filters.

The active fluids were loaded into the channel and sealed by bolting together a microfluidic device (Figure 4.1), from bottom to top, including a sample holder which fit the motorized microscope stage (Prior Scientific, H101A), a treated microscope cover glass, a treated COC channel, a polydimethylsiloxane (PDMS) layer ($\sim 1\ \text{mm}$ thickness) to provide stress relief and an aluminum plate. The package can prevent the sample from drying out for more than a week.

4.2.3 Three-dimensional scanning and two-color imaging

To build an experimental set-up with two-color imaging and three-dimensional scanning, following equipment were added to an epi-fluorescence microscope (Nikon TE 200 eclipse): a four-wavelength high power LED source (Thorlabs, DC4100), two-bandwidth optical components, an air immersion objective (Nikon CFI Plan Apo VC 20 $\times$, WD = 1 mm, NA = 0.75 or Nikon CFI S Plan Fluor ELWD 20 $\times$, WD = 6.9, NA = 0.45) held by a piezo z-drive (Physik Instrumente, Model P-725), an optical splitter (Cairn Research, Optosplit II) and an sCMOS camera (PCO edge 5.5). The microscope and associated equipment were contained inside a chamber, which maintained the temperature of the experimental environment at $25 \pm 0.3^\circ\text{C}$.

Two LEDs (405 nm and 565 nm) of the light source were energized for illumination. The excitation light, refined using a two-bandwidth excitation filter (Chroma Technology, 59003x, 395/30 nm and 575/30 nm), was directed through the air immersion objective held by the piezo z-drive and a microscope cover glass to the active sample. The fluorescently-labelled microtubules and fluorescent tracer particles in the sample emitted light at red and blue wavelengths respectively. The emission light passed through a two-bandwidth dichroic mirror (Chroma Technology, 59003bs, transmission in 460/30 nm and 630/60 nm) and was directed to an optical splitter that separated the two colors using a dichroic mirror (Chroma Technology, 565dcxr). Two light streams passed through corresponding band-pass filter (Chroma Technology, ET450/50m and D620/60m) in the optical splitter before being projected onto two halves of the sCMOS sensor. Although the full sensor size of the camera is 2560 pixels $\times$ 2160 pixels, limitations with the windows of the optical splitter result in a final image of 2500 pixels $\times$ 1300 pixels. The exposure time of the sensor was 25 milliseconds. The recording frequency was 1 Hz for the Airy disk calibration, 2.5 Hz

for validation and 10 Hz for active flows in toroidal channels. The camera settings and image writing were adjusted using CamWare64 software (PCO).

The three-dimensional scanning was accomplished by the piezo z-drive, which can hold the objective and scan up to 400 μm along the z -axis. The z-drive accepts a voltage input between $[0, 10]$ V, where 0 V represents the home position, 10 V represents the maximum position (400 μm above), and other positions are linearly related to the input voltage. During experiments, the scanning positions were provided by a controlling computer, using a digital-to-analog converter (National Instruments, DAQ PCI6052e). For the purpose of synchronization, the LED and the camera were externally triggered by the rising edge of a timing pulse (Berkeley Nucleonics Corp., Model 577), while the digital-to-analog converter was externally triggered by the falling edge. This timing ensured that the excitation and recording took place while the objective was stationary.

4.2.4 Three-dimensional particle tracking velocimetry

The three-dimensional positions were acquired by detecting the Airy disks associated with out-of-focus particles [42, 43, 44, 51]. The Airy disk radius reflects the z -position of an out-of-focus particle, and the Airy disk center determines the particle's location in the horizontal plane. Therefore, three-dimensional particle tracking is transformed into a circle detection problem.

The position and size of the Airy disks were detected using a well-developed method, the Circle Hough Transform (CHT) [52]. The detection code, written in MATLAB, followed established pattern recognition concepts [53], the CHT algorithm [45] and parallel processing method (MATLAB Image Processing Toolbox).

A reference library, which correlates the Airy disk radius with the z -location of a particle, was created by imaging a $1\text{ }\mu\text{m}$ diameter fluorescent particle, immobilized in 1% (w/w) agarose gel, over a range of $100\text{ }\mu\text{m}$ along z -axis in $0.1\text{ }\mu\text{m}$ increments. Scanned images were stacked together to form a light cone (Figure 4.2) [51].

In each frame of an experimental sequence, the three-dimensional position of detected particle was determined, as described above. After repeating the detection for every frame, a time history of each particle’s position was formed. The displacement of a given particle, between a pair of successive frames, was estimated using the “nearest neighborhood” method [56]. The velocity was calculated using a first-order finite difference.

4.2.5 Data collection and analysis

Before the data collection of confined active fluids, we performed a validation experiment for the imaging system and the three-dimensional particle tracking. Details about the accuracy of measurements are discussed in Appendix D.

During the experiments of assembled active systems, the scanning system was deactivated in order to pursue a better temporal resolution. Image recording focused on a $350\text{ }\mu\text{m} \times 350\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$ observation region at corresponding center of most confining geometries (Figure 4.3) and was initiated within 200 seconds since the active fluids were assembled. The “**” note in Table 4.1 identifies a special case that focal plane was 0.2 mm below the geometrical center, due to the restriction from the working distance of the objective. For each channel, experiments were repeated twice.

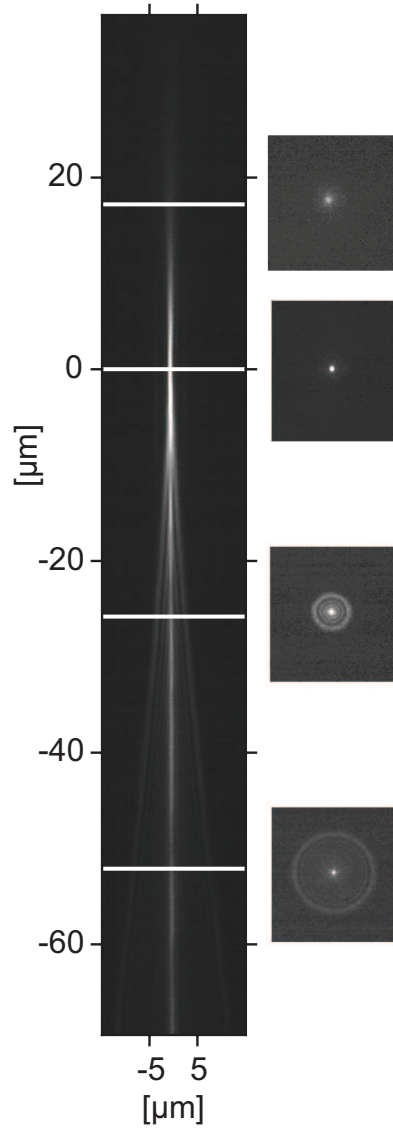


Figure 4.2: A light cone of a trapped fluorescent particle. 0 represents the z -position where the particle is in focus. Positive and negative value indicate above and below the focal plane respectively. Airy disks are shown by horizontal slices at four z -positions.

In this chapter, data analysis only focuses on one color in the active fluids, which is the blue fluorescent particle. Results from fluorescent microtubules will be addressed separately.

A complete data set, recording active flows in a COC channel, consists of N images, taken at a constant frequency, $f = 1/\Delta t_o$, where Δt_o is the temporal resolution of image recording, typically 0.1 seconds. Each image, corresponding to time $t_k = k\Delta t_o$ ($k = 1 \dots N$), has P_k particles, and each particle in that image, denoted by the index j ($j = 1 \dots P_k$), has a position:

$$\mathbf{X}^j(t_k) = (x^j(t_k), y^j(t_k), z^j(t_k)). \quad (4.1)$$

Each particle is tracked from frame to frame, and from this time history of particle positions, the velocity vector of the individual fluorescent tracer particle is defined as

$$\mathbf{u}^j(t_k) = (u_x^j(t_k), u_y^j(t_k), u_z^j(t_k)), \quad (4.2)$$

where

$$\begin{aligned} u_x^j(t_k) &= \frac{x^j(t_{k+1}) - x^j(t_k)}{\Delta t_o}, \\ u_y^j(t_k) &= \frac{y^j(t_{k+1}) - y^j(t_k)}{\Delta t_o}, \\ u_z^j(t_k) &= \frac{z^j(t_{k+1}) - z^j(t_k)}{\Delta t_o}. \end{aligned} \quad (4.3)$$

We define specific kinetic energy, e , as the kinetic energy per unit mass, and for this discussion, we divide the energy to components parallel to and perpendicular to confining surfaces:

$$e(t) = \frac{E(t)}{\frac{1}{2}m} = e_{\parallel}(t) + e_{\perp}(t) = [u_x^2(t) + u_y^2(t)] + u_z^2(t). \quad (4.4)$$

Since the system is homogeneous over the image, we can average over both the number of particles, P_k , in the image at each time, t_k , and over a time period $T = [t_s, t_e]$, where

$$t_e = t_s + N\Delta t_o. \quad (4.5)$$

Following the standard turbulence literature (for example [68]), we employ the “Reynolds decomposition” for the velocity (and other quantities):

$$u_i(t_k) = \bar{u}_i + u'_i(t_k) \quad (i = x, y, z), \quad (4.6)$$

where the average velocity, $\bar{u}_i$, denotes a temporal average over N images:

$$\bar{u}_i = \frac{1}{N} \sum_{t_s}^{t_e} \frac{1}{P_k} \sum_{j=1}^{P_k} u_i^j(t_k). \quad (4.7)$$

The variance of the fluctuating velocity, $\overline{u_i'^2}$, is defined as:

$$\overline{u_i'^2} = \sigma_i^2 = \frac{1}{N} \sum_{t_s}^{t_e} \frac{1}{P_k} \sum_{j=1}^{P_k} (u_i^j(t_k) - \bar{u}_i)^2. \quad (4.8)$$

For all of the experiments discussed here, the statistics are calculated from a time period covering one-hour relatively stabilized activity of the active systems, from $t_s = 2000$ seconds to $t_e = 5600$ seconds ($k = t_s \cdot f, (t_s + 1) \cdot f, \dots, t_e \cdot f$).

As with the specific kinetic energy, we compute mean square displacement (MSD) of traced particles in a plane parallel to and along an axis perpendicular to the

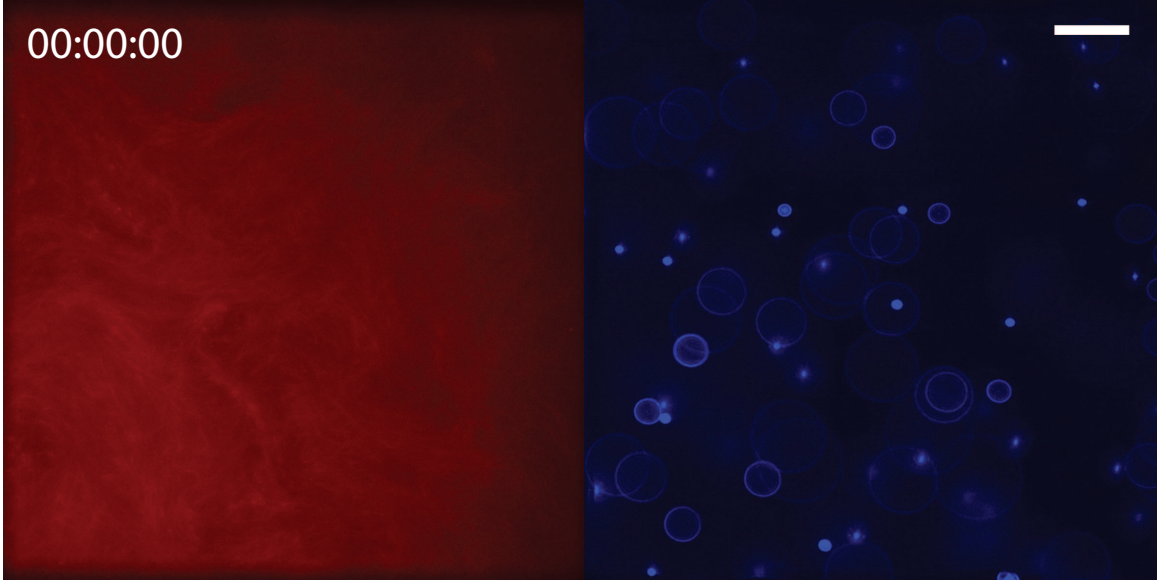


Figure 4.3: An image frame of two-color active fluids in a COC channel ($H = 0.1$ mm) at the central level, z , where $z = 50$ μm . Red window shows microtubule bundles and blue window shows tracer particles. Scale bar: 50 μm .

confining boundary:

$$\begin{aligned}
 \langle d_{\parallel}^2(\Delta t) \rangle &= \frac{1}{P} \sum_{j=1}^P \left((x^j(t + \Delta t) - x^j(t))^2 + (y^j(t + \Delta t) - y^j(t))^2 \right), \\
 \langle d_{\perp}^2(\Delta t) \rangle &= \frac{1}{P} \sum_{j=1}^P (z^j(t + \Delta t) - z^j(t))^2.
 \end{aligned} \tag{4.9}$$

Here, the angle brackets, $\langle \dots \rangle$, denote an ensemble average over all particles P , collected from N images during the time period $T = [t_s, t_e]$. To normalize by variations in activity (which varies depending on the confinement), the MSDs are scaled by a product of velocity variance (Equation 4.8) and temporal resolution square: $(\sigma_x^2 + \sigma_y^2)\Delta t_o^2$ for the parallel plane and $\sigma_z^2\Delta t_o^2$ for the perpendicular axis.

Temporal correlations are defined by velocity-velocity correlations parallel to and

perpendicular to the confining boundary surface, as with mean square displacement:

$$\begin{aligned} R_{\parallel}(\Delta t) &= \frac{\langle \mathbf{u}_{\parallel}^j(t) \cdot \mathbf{u}_{\parallel}^j(t + \Delta t) \rangle}{\langle \mathbf{u}_{\parallel}^j(t) \cdot \mathbf{u}_{\parallel}^j(t + \Delta t_o) \rangle}, \\ R_{\perp}(\Delta t) &= \frac{\langle \mathbf{u}_{\perp}^j(t) \cdot \mathbf{u}_{\perp}^j(t + \Delta t) \rangle}{\langle \mathbf{u}_{\perp}^j(t) \cdot \mathbf{u}_{\perp}^j(t + \Delta t_o) \rangle}. \end{aligned} \quad (4.10)$$

Here, $\mathbf{u}_{\parallel}^j(t)$ is the horizontal velocity vector of j -th particle, parallel to the confining surface and $\mathbf{u}_{\perp}^j(t)$ is the vertical velocity vector of j -th particle, perpendicular to the confining surface:

$$\begin{aligned} \mathbf{u}_{\parallel}^j(t) &= (u_x^j(t), u_y^j(t), 0), \\ \mathbf{u}_{\perp}^j(t) &= (0, 0, u_z^j(t)), \end{aligned} \quad (4.11)$$

and the normalization is with respect to the shortest separation time Δt_o . Since this is a Lagrangian correlation, we are able to track particles over a time period, Δt , in all three dimensions, and we can compute the wall-normal temporal correlation as well as the parallel correlation.

Spatial correlations in the plane parallel to confining boundary during the time period, $T = [t_s, t_e]$, are defined as:

$$C_{\parallel}(\Delta r) = \frac{\langle \mathbf{u}_{\parallel}(\mathbf{r}, t) \cdot \mathbf{u}_{\parallel}(\mathbf{r} + \Delta r, t) \rangle}{\langle \mathbf{u}_{\parallel}(\mathbf{r}, t) \cdot \mathbf{u}_{\parallel}(\mathbf{r} + \Delta r_o, t) \rangle}. \quad (4.12)$$

As before, $\mathbf{u}_{\parallel}(\mathbf{r}, t)$ is the horizontal velocity vector, where

$$\mathbf{u}_{\parallel}(\mathbf{r}, t) = (u_x(\mathbf{r}, t), u_y(\mathbf{r}, t)), \quad (4.13)$$

and $\mathbf{r}$ is the coordinate vector in the horizontal plane:

$$\mathbf{r} = (x, y). \quad (4.14)$$

Here Δr is a spatial separation in the horizontal plane:

$$\Delta r = \sqrt{\Delta x^2 + \Delta y^2}. \quad (4.15)$$

From a practical perspective, the correlations are binned so that correlations from a small range of separations are averaged together. The spatial correlations are normalized by the correlation at some small, but non-zero separation, Δr_o (typically 30 microns). Because of the finite field of view in the $x - y$ plane, the correlation functions do not generally extend to large enough distances to decay to zero. Consequently, in order to estimate a correlation length, l_c , we fit an exponential function to the correlation function:

$$C_{\parallel}(\Delta r) \approx Ae^{b\Delta r}, \quad (4.16)$$

and integrate the fitted function, yielding $l_c = -A/b$. Since the average tracking range in z -axis is only $\sim 40 \mu\text{m}$, a meaningful wall-normal Eulerian spatial correlation cannot be calculated.

4.3 Results and discussion

4.3.1 Lifetime and activity of active flows

Since we only have in-plane tracking for the largest channel (Table 4.1), we quantify the activity of the active fluids using the parallel component of specific kinetic energy, $e_{\parallel}$ (Equation 4.4). This parallel component demonstrates the same pattern for all active systems: evolving quickly to a relatively stable state, remaining approximately constant before eventually dropping after ~ 3 hours (Figure 4.4A). The lifetime of

activity depends on the consumption rate of ATP which, in turn, solely relies on the concentration of kinesin motor clusters in the volume and their ability to bind to and walk along microtubules. The fact that activity lifetime is unaffected by channel geometry confirms that the specific chemical energy consumption rate, which is associated with the motion of molecular motor clusters and occurs at the smallest scales in active fluids, remains constant and independent of the system confinement.

In contrast to the consistent lifetime and chemical energy consumption rate, Figure 4.4A indicates that there is a clear correlation between the system confinement and the activity of active fluids, which is reflected by the average level of specific kinetic energy and its fluctuation amplitude. Unlike the toroidal channel systems which can show a sustained coherent average velocity [28], none of the cuboid systems considered here exhibits a mean velocity (Figure 4.4B - circles). The fluctuating strengths (Figure 4.4B - squares) exhibit a strong dependence on the system size, rising from approximately $3 \mu\text{m/s}$ for the smallest system ($H = 0.1 \text{ mm}$) to approximately $6 \mu\text{m/s}$ for the largest system considered, $H = 4 \text{ mm}$. The fluctuating strengths initially rise linearly with the channel height and show equivalent amplitudes among all axes in each confinement. However, the amplitude seems to saturate at the largest channel and appears to be levelling off by $H = 4 \text{ mm}$. Since the confining walls are hundreds of microns from the measured volume (Table 4.1), this isotropy is to be expected at the largest scale. That the fluctuations remain isotropic at the strongest level of confinement reflects the fact that these velocity fluctuations are generated by motion occurring at the smallest, most local scale — that of one microtubule filament sliding past another. The persistence of isotropy suggests that the microtubule motion is randomly oriented, even as the confinement scale becomes closer to the estimate microtubule fragment size of approximately 20 microns [27].

In addition to the dependency of the overall amplitude of the velocity fluctuations, Figure 4.4A indicates that there is a clear scale dependence in the amplitude excursions and the temporal scale of the velocity fluctuations. At the smallest scale, the turbulence level is very consistent while in the larger systems, the fluctuation levels and their characteristic time scales appear to grow as the system scale grows. These qualitative observations will be characterized more carefully in the following sections using spatial and temporal velocity correlations.

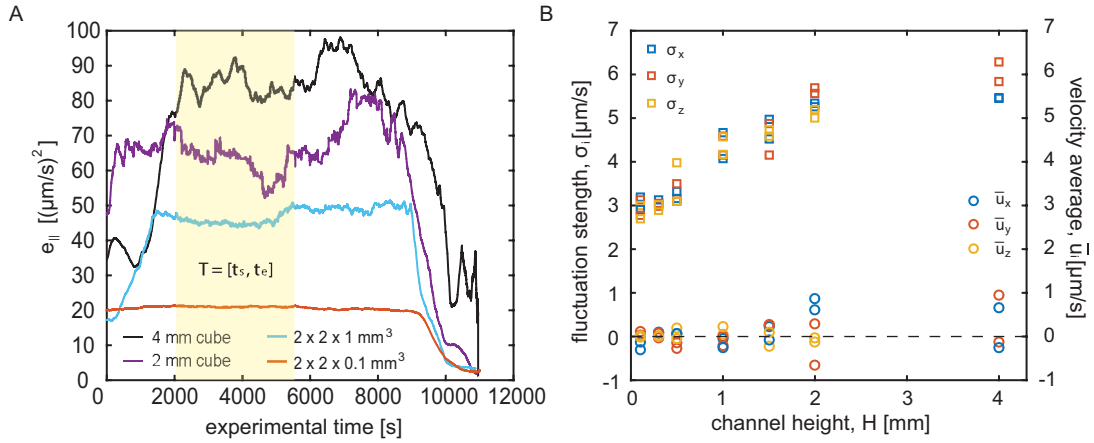


Figure 4.4: (A) Lifetime of active systems in varying confinement. Parallel component of specific kinetic energy, $e_{\parallel}$, is shown. The highlighted area defines a time period, $T = [t_s, t_e]$, over which temporal averaging takes place. (B) Mean and fluctuation velocity of active fluids as a function of confinement. Averaged velocity, $\bar{u}_i$, (over time period T) in circle and velocity fluctuation strength, σ_i , in square mark. Repeated symbols represent duplicate experiments in identical channels.

4.3.2 Mean square displacements

The mean square displacements (MSDs) of particles (Equation 4.9) are used to explore the flow structure at longer timescales and to reveal the aspects of flow regime, by examining the exponent of the MSD as a function of time. When a MSD has an exponent of 2, flow is termed “ballistic”, implying advective transport [41]. For the exponent between 1 and 2, flow is convective, and can also be referred as super-diffusion [77, 78]. If the exponent is 1, then particles are performing diffusive (Brow-

nian) motion [79]. With an exponent smaller than 1, the flow is sub-diffusive, which can be observed in restrained diffusive motion near a solid boundary, caused by an anisotropic drag force [80, 81].

Figure 4.5A shows the scaled in-plane MSD:

$$MSD_{\parallel} = \frac{\langle d_{\parallel}^2(\Delta t) \rangle}{(\sigma_x^2 + \sigma_y^2)\Delta t_o^2}, \quad (4.17)$$

where the scaling is chosen to account for the variations in the fluctuation levels associated with each confining geometry. Ballistic motion is observed for all time lags when the channel height is greater than one millimeter. As the height decreases, a transition from diffusive to super-diffusive behavior appears. In a similar manner of scaling, Figure 4.5B shows the perpendicular component of the scaled MSD,

$$MSD_{\perp} = \frac{\langle d_{\perp}^2(\Delta t) \rangle}{\sigma_z^2\Delta t_o^2}. \quad (4.18)$$

In the 2 mm cubic channel, ballistic motion is observed even at the shortest time lag of 0.1 seconds. In the more confined geometries, sub-diffusive behavior is observed in $MSD_{\perp}$ at shorter time lags, and this behavior is observed to extend to successively longer time lags as the channel height decreases. The dashed lines in Figure 4.5A and B illustrate the crossover time, τ , defined as the time lag when the MSD slope changes from less than one to greater than one. Figure 4.5C presents the value of τ as a function of channel height ($H = 2$ mm and $H = 1.5$ mm geometries are beyond the temporal resolution). The crossover time reveals the average sub-diffusing time of particles before they are advected by sliding microtubule bundles. These transitions of MSDs can be caused by the constraints on sliding motion of microtubule bundles from confining surfaces. The constraints are initiated along the axis normal to confining surfaces and then spread to three dimensions as the level of confine-

ment increases. In living murine fibroblast cells at room temperature, mean square displacements of nano-scale particles show similar transitions from sub-diffusive to super-diffusive or ballistic behavior [82]. The sub-diffusive motion reflects the spatial constraints from micro-domains within the cell, potentially created by cytoskeletal network. The super-diffusive (or ballistic) behavior reveals the directional flow of particles caused by cell crawling motion at a speed of several nanometers per second. In the bundled active microtubule network, similar transitions of MSD were observed but they reflected different dynamics in the system caused by decreasing ATP concentration or decreasing depletion polymer concentration [27, 34].

We observed a decreasing exponent of $MSD_{\perp}$ from 2 mm cubic channel (Figure 4.5B) at longer time lags and there are two potential explanations. The offset of the objective focal plane, which is 0.2 mm away from the central z -level (Table 4.1), contributes to the decreasing exponent, since the observation volume is closer to one planar boundary and is only 50 μm different from the central level in $H = 1.5$ mm channel. Another opinion, the decreasing exponent of $MSD_{\perp}$ as the function of time lag indicates the average lifetime of collective motion in active flows because at longer time lags, all MSDs should become diffusive. However, due to the restriction of the imaging system which limits the maximum time a particle can be followed, we were not able to track particles for longer periods. In dilute bacterial suspension, the long-time tracking of individual bacterium or tracer particle allows the calculation of mean square displacement at long time lags over ten seconds [41] or even more than a hundred seconds [40]. The crossover times from ballistic to diffusive behavior indicate that the average run-time of wild type bacteria in the suspension is between 1 to 2 seconds [40, 41].

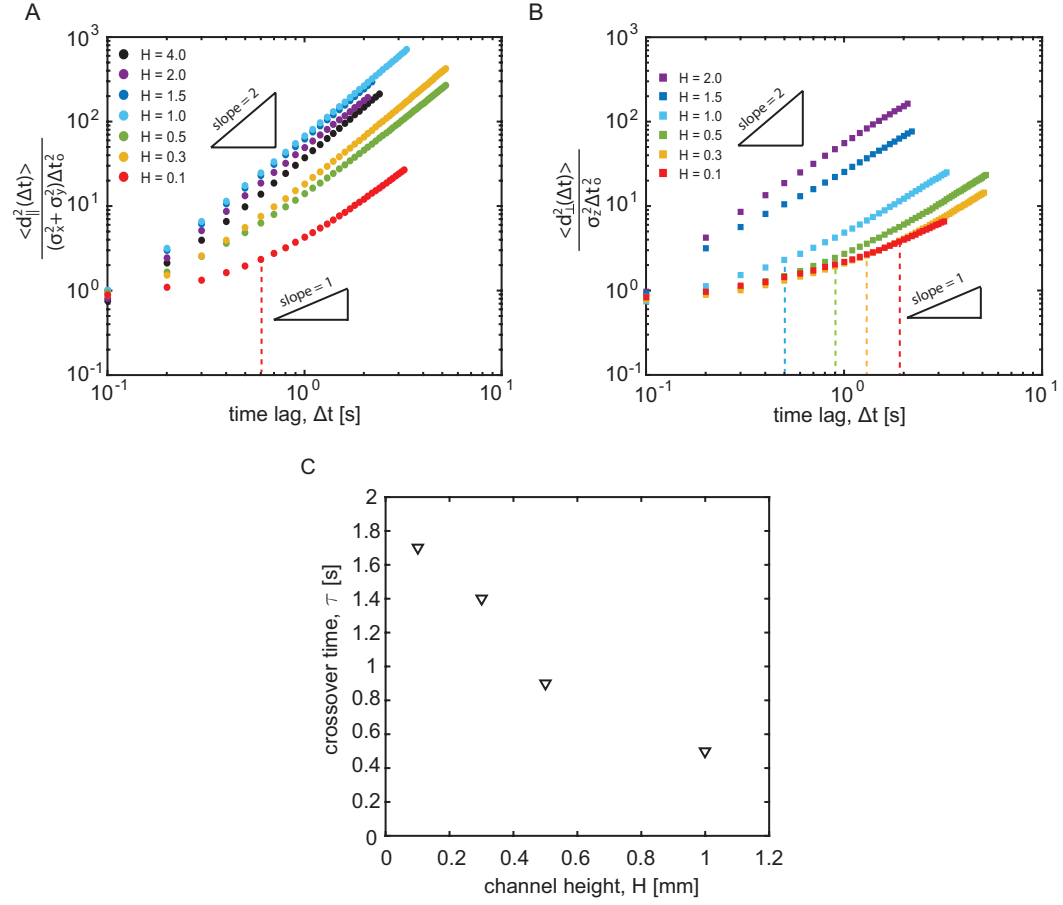


Figure 4.5: (A) Scaled mean square displacements of fluorescent particles in $x - y$ plane as a function of time lag for different confining geometries. Triangles indicate ballistic exponent of 2 and diffusive exponent of 1. Repeated experiments from the same geometry are averaged together. (B) Scaled mean square displacements of fluorescent particles in z -axis as a function of time lag for different confining geometries. Dash line indicates crossover time τ , defined as the transition of scaled MSD in z -axis from sub-diffusion to super-diffusion. (C) Crossover time τ as a function of channel dimension.

4.3.3 Temporal correlations

Figure 4.6 shows the temporal (Lagrangian) self-correlations (Equation 4.10) of tracer particles, which reflect the “memory” of the active flow. In the plane parallel to confining boundaries (Figure 4.6A), the velocity correlation remains at a high level when the height, H , is larger than 1 mm, indicating that active flows have strongly correlated “rivers” that continue with a primary direction and magnitude for a long time. However, note that the tracking time is still relatively short, only three seconds for most systems studied. This result is consistent with the ballistic nature of the in-plane MSD (Figure 4.5A), which implies advective flows. As the confining height decreases, we see that the parallel component of the temporal correlation, $R_{\parallel}(\Delta t)$, transitions rather abruptly to one that decays very quickly; initially, for $H = 0.5$ mm and 0.3 mm, the correlation drops to a value around 0.2 after only 0.5 seconds time lag, while for the smallest value of H studied, $H = 0.1$ mm, the correlation drops below zero and then fluctuates about zero for the entire time range studied.

The perpendicular temporal correlation, $R_{\perp}(\Delta t)$, (Figure 4.6B) shows a somewhat similar behavior to the parallel correlation. However, in this direction, even the larger scale systems, $H = 2$ and 1 mm show a relatively quick decay in the correlation coefficient, and the more confined systems illustrate a very rapid decay, and hover around zero for all times greater than 0.5 seconds.

The negative correlation in the parallel correlation at $H = 0.1$ mm may or may not be significant, since it is only observed for a single value of Δt . However, the short dip below zero is consistently observed in several of the perpendicular correlations and so one is tempted to believe that these negative correlations are real, indicating the existence of a “vortex-like-structure” in which the particle direction reverses

within about 0.5 seconds. In the present case, at an average velocity of a few microns per second (Figure 4.4), this might represent a structure of only a few microns in diameter, which may be associated with the opposing motion of tracer particles on either side of a sliding microtubule bundle. At the larger system scales, $H > 0.3$ mm, larger collective flows appear to become established (hence the ballistic MSDs observed in Figure 4.5) and these “rivers” overwhelm the small vortical flows associated with individual microtubule motion. Similar temporal correlations were observed in confined dense bacteria suspensions, which showed vortex structures [83, 84]. The correlation drops in a short period and fluctuates around zero for longer time lag. The size of the “vortex-like structure” in a bacterial suspension, from a few microns to several dozens of micrometers, was dependent on the size of single bacterium and the geometries of confining chambers [83, 84, 85].

One curious feature of the Lagrangian correlations is the sudden transition observed in the parallel correlation function, $R_{\parallel}(\Delta t)$ as the scale drops from $H = 1$ mm to 0.5 mm. This may be indicative of a sharp instability boundary, or phase transition boundary. For larger systems, larger spiral flows might be able to be sustained, giving rise to the long Lagrangian correlations (Figure 4.6), the largely ballistic MSDs (Figure 4.5) and the much more active quality of the energy fluctuations (Figure 4.4A). For smaller scales, these motion may not be sustainable, reflected by the much more rapid fall in the temporal correlations, the increased regions of diffusive behavior and the more quiescent nature of the energy fluctuations observed. If such an instability boundary exists, one would expect that it depends on parameter other than system scale, for example, the average length of the microtubule fragments, ATP concentration, etc. This will need to be explored further in future experiments, and coordinated with on-going theoretical studies of these active systems [86, 87].

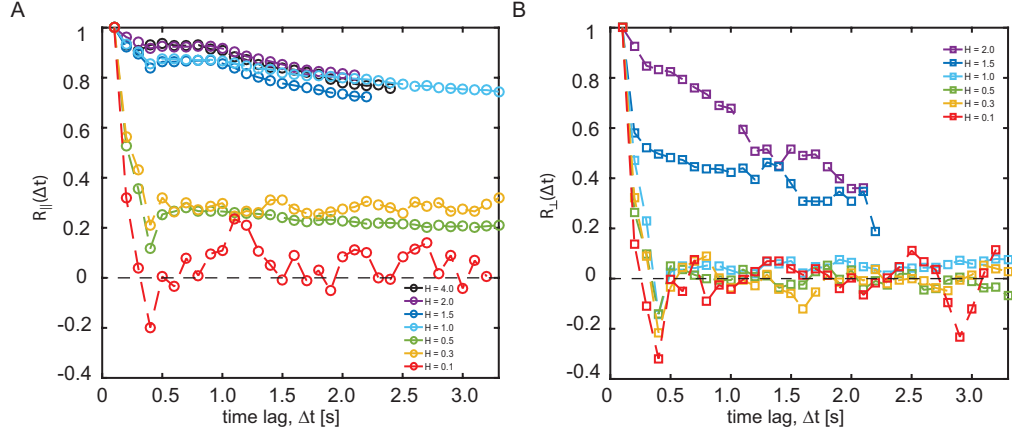


Figure 4.6: (A) Velocity-velocity temporal correlation in $x-y$ plane as a function of time lag, which is limited by the shortest tracking period of particles. (B) Velocity-velocity temporal correlation in z axis as a function of time lag. Colors are consistent with Figure 4.5.

4.3.4 Spatial correlations

Spatial correlation functions in plane parallel to confining boundary (Equation 4.12) are illustrated in Figure 4.7A with exponential fittings (solid lines). The long-ranged spatial correlation decays exponentially in large geometries and show some hints of anti-correlation in small geometries. Although as with the temporal correlations, the negative correlations are weak. The exponential decay seems to fit well for all confinement levels, and we can use these fits from repeated experiments in each geometry to estimate a correlation length, l_c (Figure 4.7B). The estimated length, l_c , which reveals a characteristic structure size in the active microtubule networks, increases with channel height, H , and appears to saturate in the largest system, $H = 4$ mm. The existence of an upper limit in the correlation length implies that there exists a maximum possible structure size, around $400 \mu\text{m}$, which is two orders of magnitude larger than the average microtubule length, $1 \mu\text{m}$ [28], and still one order of magnitude smaller than the dimension between confining walls. In similar microtubule-based active systems, the anti-correlation is reached around $200 \mu\text{m}$ for a quasi-2D confinement [27]. Here, we see an anti-correlation at around $170 \mu\text{m}$ in the thinnest channel, indicating a similar structure size. Though we have no access

to meaningful wall-normal spatial correlations due to the restricted average tracking range in z -axis with one static focal plane, the geometries of confinement and spatial correlations in parallel plane provide enough information. The estimated correlation length from $C_{\parallel}(\Delta r)$ reveals a projection of the flow structure to the $x - y$ plane. Therefore, our results show that the characteristic size of the three-dimensional flow structure varies with the smallest dimension of the confining geometry.

So far, similar large-scale structures have been observed in a wide variety of confined active materials, including cytoskeletal extracts [35, 64] and cells or cell sheets [10, 11]. Microtubule-based cytoskeletal extracts form coherent structures at length scales from 100 to 700 μm , which are larger compared to our results due to the longer average length of microtubule filaments (around 15 micrometers) [64]. The large structures observed in cells are between 50 to 100 μm and similarly, there exists a saturation level (approaching 100 μm) as the confinement geometry increases [11]. In general, the characteristic size of self-organized structure is highly dependent on confinement geometry and intrinsic length scale of the active system. Although activity is observed at microscopic scale at very low Reynolds number ($Re \sim 10^{-9}$), the chaotic dynamical state shows a statistical similarity as the isotropic turbulence at high Reynolds number [88]. The spatial correlation functions of the isotropic turbulence, after normalization, show the same profiles as Figure 4.7A [89, 90, 91]. The statistical analysis, adopted from the isotropic turbulence theory, may provide a good way to further investigate of the kinesin-driven active fluids.

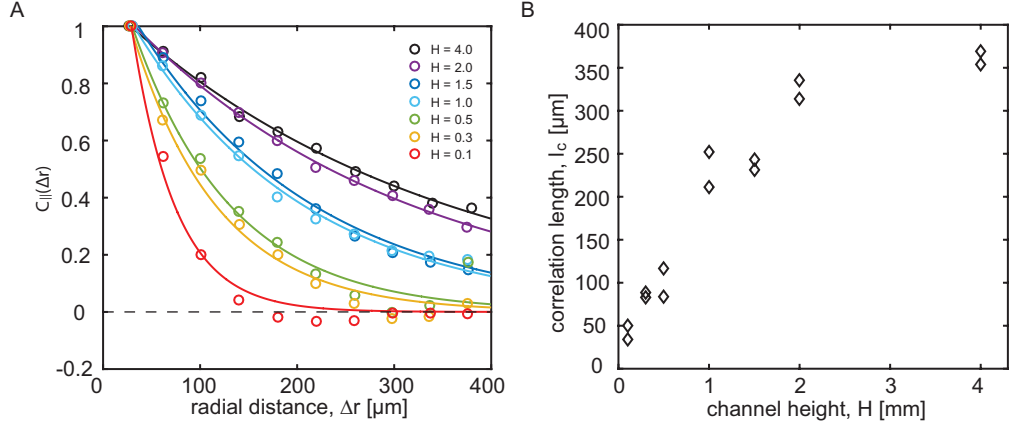


Figure 4.7: (A) Normalized equal-time spatial velocity-velocity correlation as a function of separation distance for varying confinements. Equal time interval is $\Delta t_o = 0.1$ s. Circles denote spatial correlation functions from experiments and solid lines present exponential fittings to experimental data. (B) Correlation length, l_c , as a function of confining geometry. Repeat mark represents duplicate experiments in identical geometry.

4.4 Conclusions

The “turbulent-like” dynamics of microtubule-based active fluids show many interesting features that we are only just beginning to understand. At the shortest timescale, the level of activity, measured from the kinetic fluctuations of passive tracers increases with the system size and maintains isotropy at every level of tested confinement. However, the nature of those kinetic fluctuations is strongly dependent on the proximity of confining walls. In an unconfined system (or as close as we can get with the current experimental setup), there appear to exist large-scale collective flow structures, with sizes of order 400 microns, that advect particles. As the system becomes confined in one direction, the large-scale isotropy is quickly lost, with the wall-normal temporal correlations decaying faster than the equivalent correlations in the homogeneous direction, and the mean square displacements showing more persistent diffusive behavior at short timescales.

There is evidence, from the character of the energy fluctuations and the temporal correlations, that there may be a sharp transition between the large-scale collective

motion and the chaotic flows and that, for a given set of operating parameters (microtubule filament size, concentrations of depletion agents, ATP, etc.) there might exist a critical channel size below which larger-scale structures are not stable.

Several more experiments, beyond the scope of this thesis, will help to elucidate these issues. The present experiments were all conducted at, or close to, the geometric center of the system. One natural series of measurements is to look at the statistics of these confined systems at other locations - closer to the confining walls. A second series of measurements would be to look at the microtubule motion and bundle orientation as a function of confinement (at different locations in the channel). These, coupled with emerging theories of active systems, will lead to a stronger, more comprehensive understanding of the mechanisms behind natural scale selection and pattern formation that govern the dynamics of these innovative active fluid networks.

CHAPTER FIVE

Interaction between active microtubules and passive particles

5.1 Velocity field of kinesin-driven microtubule bundles

The velocity analysis of active microtubule bundles is inspired by particle imaging velocimetry (PIV) [56, 58]. Following the PIV analysis, a two-dimensional displacement field can be obtained over the entire field of view. The displacement field is converted to an Eulerian velocity field using the temporal resolution of every image pair depending on the camera recording frequency. The same process is repeated over thousands of locations in all images acquired from multiple z -positions.

The basic principle of displacement detection in PIV analysis is to search for the maximum of an intensity convolution function from each interrogation window. Suppose that two sub-images, from a pair of images at an instant time, t , and a following time, $t + \delta t$, have intensities, I_1 and I_2 , in an identical interrogation window centered at a location, $\mathbf{X}$:

$$\begin{aligned} I_1(\mathbf{X}) &= I(\mathbf{X}, t), \\ I_2(\mathbf{X}) &= I(\mathbf{X}, t + \delta t). \end{aligned} \tag{5.1}$$

The intensity convolution, also called as spatial cross-correlation function, is defined as

$$\mathcal{R}(\mathbf{s}) = \int_{\mathcal{W}} I_1(\mathbf{X}) I_2(\mathbf{X} + \mathbf{s}) d\mathbf{X}, \tag{5.2}$$

where $\mathbf{s}$ is the separation vector and $\mathcal{W}$ refers to the interrogation window. If the displacement vector between I_1 and I_2 is $\Delta\mathbf{X}$, then the maximum of integrand occurs when $\mathbf{s} = \Delta\mathbf{X}$, where $\Delta\mathbf{X} = (\Delta x, \Delta y)$. As a consequence, locating the peak of the convolution, $\mathcal{R}$, ultimately determines the displacement between two sub-images. Objects do not all move at the same velocity so that the displacement, found by

convolution in Equation 5.2, is an average over an interrogation window, $\mathcal{W}$, or also known as a region of interest. The size of $\mathcal{W}$, $\mathcal{N} \mu\text{m} \times \mathcal{N} \mu\text{m}$, is determined based on a typical PIV analysis that each interrogation window contains about 10 – 15 particles (10 – 15 microtubule filaments here) and neighbor windows are set to have 2/3 overlapping area [58]. An image is divided into multiple interrogation windows and the total number of displacements in the field of view equals to the number of windows. With the temporal resolution of image recording and number of scanning positions, we obtain a velocity field (Eulerian) of microtubules at time t :

$$\mathbf{u}_m(\mathbf{X}, t) = (u_m(\mathbf{X}, t), v_m(\mathbf{X}, t)), \quad (5.3)$$

where $\mathbf{X}$ presents centers of interrogation windows. Figure 5.1 shows velocity fields of active microtubule bundles in chaotic dynamical state from PIV analysis (LaVision, DaVis software) with two different interrogation window sizes as $\mathcal{N} = 15 \mu\text{m}$ (Figure 5.1A) and $\mathcal{N} = 30 \mu\text{m}$ (Figure 5.1B). With the smaller interrogation window, detected mean speed is $0.44 \mu\text{m/s}$ over the field of view and maximum speed is $7.4 \mu\text{m/s}$. With the larger interrogation window, detected mean speed is $0.45 \mu\text{m/s}$ over the field of view and maximum speed is $6.9 \mu\text{m/s}$. Each velocity vector, from the $30 \mu\text{m}$ square interrogation window, is compared with the average of a corresponding two-by-two velocity vector matrix, from the $15 \mu\text{m}$ interrogation window. The variation averaged from all velocity vectors (1520 vectors) in the field of view is 2.1%. Therefore, velocity field is not sensitive to interrogation window size between $15 \mu\text{m}$ to $30 \mu\text{m}$. We repeat the same analysis with $15 \mu\text{m}$ interrogation window size for every consecutive image pairs and obtain a velocity field with a history over experimental time at each z -position.

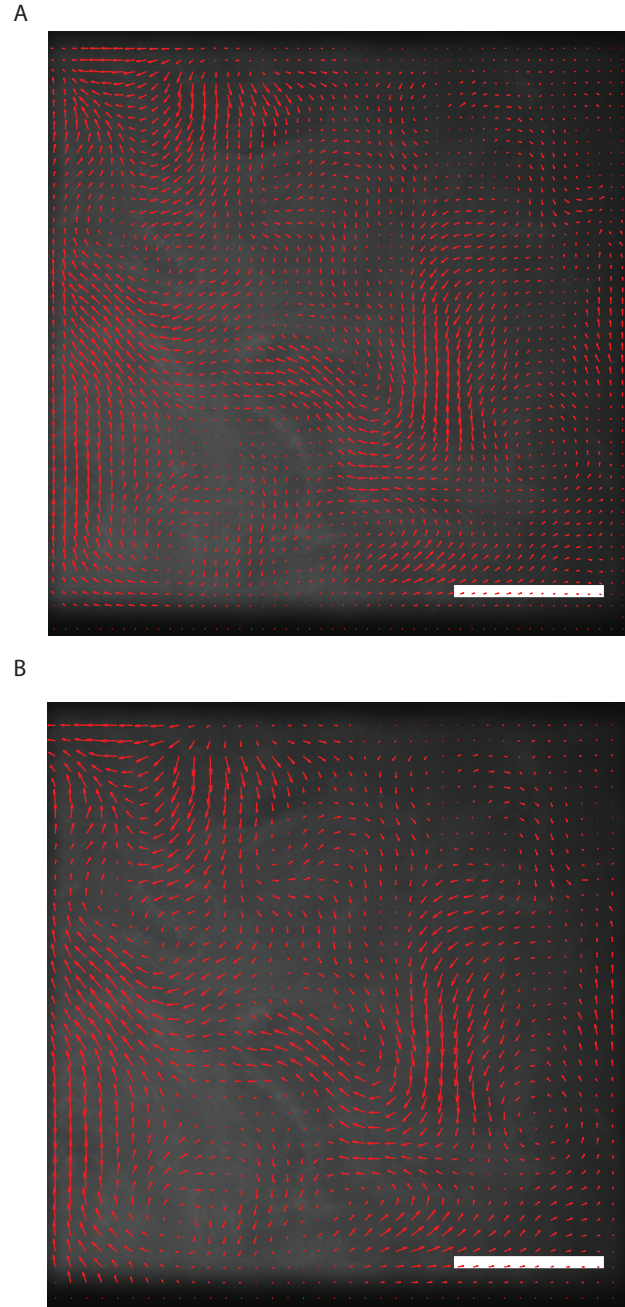


Figure 5.1: Velocity field of active microtubule bundles from PIV cross-correlation analysis. Arrows indicate local bundle velocity. Scale bars: $100\ \mu\text{m}$. (A) Interrogation window with a square dimension of $15\ \mu\text{m} \times 15\ \mu\text{m}$. (B) Interrogation window with a square dimension of $30\ \mu\text{m} \times 30\ \mu\text{m}$.

5.2 Two-dimensional particle tracking velocimetry

In order to study the interaction between microtubules and particles, we reduce the dimensionality of particle tracking from three dimensions to two dimensions by only tracking in-focus particles. As a consequence, the radius of individual particle within the focal plane is detected through the Circle Hough Transform (CHT) [45, 52], but only its center location is used for displacement analysis in $x - y$ plane. The target range of radii for CHT is $(0, 2) \mu\text{m}$ (Figure 5.2). The centers of detected in-focus particles are obtained using a circular fitting model [55]. The displacements in $x - y$ plane are estimated using the “nearest neighborhood” method with a circular interrogation window [56]. In each interrogation window, similar to velocity calculation in Chapter 2, there are three outcomes:

1. No particle is found in the following frame, which means we lose track of a particle;
2. One particle is detected solely in the interrogation circle of the original particle, in which case we link both particles, and calculate the displacement in the x and y axes respectively;
3. More than one particle is detected. In this case, we apply two conditions: that each particle cannot be linked more than once, and that each particle prefers to be linked to the one with the shortest separation, known as the “nearest neighborhood” method in particle tracking velocimetry.

In this manner we are able to identify displacement of each particle from frame to frame, and via dividing by the temporal resolution, δt , we can define a particle

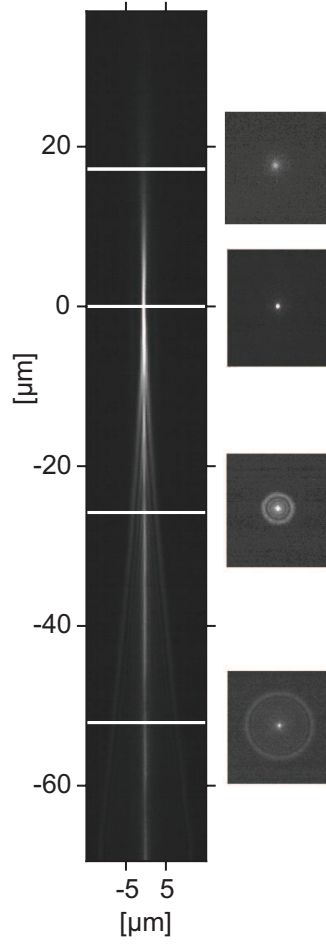


Figure 5.2: Stacked images of Airy disks of an immobilized fluorescent particle ($1\ \mu\text{m}$ diameter). 0 represents the focal plane. Positive and negative value indicate above and below the focal plane respectively. When the radius of the Airy disk is in the range of $(0, 2]\ \mu\text{m}$, the particle is treated as in-focused.

velocity. With a collection of in-focus particles, velocity fields (Lagrangian) within the focal plane are obtained.

5.3 Data analysis

From the two-dimensional particle tracking, the location of each particle, $\mathbf{X}_p$, is varying as a function of time, t :

$$\mathbf{X}_p^j(t) = (x_p^j(t), y_p^j(t)). \quad (5.4)$$

Here, j is the index of particle. Based on the displacement analysis from the “nearest neighbor” method and the temporal resolution, δt , we have a Lagrangian velocity field of a single particle as

$$\mathbf{u}_p^j(t) = (u_p^j(t), v_p^j(t)), \quad (5.5)$$

where $\mathbf{u}_p^j(t)$ is the velocity of j -th particle at time t , calculated as

$$\begin{aligned} u_p^j(t) &= \frac{x_p^j(t + \delta t) - x_p^j(t)}{\delta t}, \\ v_p^j(t) &= \frac{y_p^j(t + \delta t) - y_p^j(t)}{\delta t}. \end{aligned} \quad (5.6)$$

The two sub-frames of the optical splitter are registered for the same observation region during calibration process in Chapter 2, therefore, the location of corresponding microtubule is

$$\mathbf{X}_m^j(t) = (x_m^j(t), y_m^j(t)), \quad (5.7)$$

where

$$\begin{aligned} x_m^j(t) &= mx_p^j(t) + ny_p^j(t) + q, \\ y_m^j(t) &= m'x_p^j(t) + n'y_p^j(t) + q', \end{aligned} \quad (5.8)$$

where m, n, q and m', n', q' are mapping constants obtained from the registration of the optical splitter. The interaction is examined by velocity-velocity correlations between particles and their neighbor microtubules as

$$C_{pm} = \frac{\langle \mathbf{u}_p(\mathbf{X}_p) \cdot \mathbf{u}_m(\mathbf{X}_m + \Delta s) \rangle}{\langle \mathbf{u}_p(\mathbf{X}_p) \cdot \mathbf{u}_m(\mathbf{X}_m) \rangle}, \quad (5.9)$$

where Δs is a small separation distance (typically less than $20 \mu\text{m}$) between corresponding microtubule (mapped from the location of a detected in-focus particle) and neighbor microtubule bundles, defined as

$$\Delta s = \sqrt{\Delta x^2 + \Delta y^2}. \quad (5.10)$$

Here, the angle brackets, $\langle \dots \rangle$, denote an ensemble average over all particles detected from images during the defined period, T ($T = 2000$ seconds for coherent flows data from Chapter 3 and $T = 3600$ seconds for bulk flows data from Chapter 4).

5.4 Results

We revisit data from Chapter 3 and Chapter 4 to study the interaction between active microtubules and neighbor passive tracer particles. The channel geometries of data that have been analyzed are shown in Table 5.1. Figure 5.3 shows the in-plane location of a single particle (blue cross) and the location of the mapped microtubule bundle (red cross), calculated based on Equation 5.8. The velocity vectors of neighbor microtubule bundles within a separation distance, Δs , less than $20 \mu\text{m}$ (yellow circle) are used to calculate the velocity-velocity correlation (Equation 5.9). Figure 5.4 shows the correlation functions, indicating that local interaction between particles

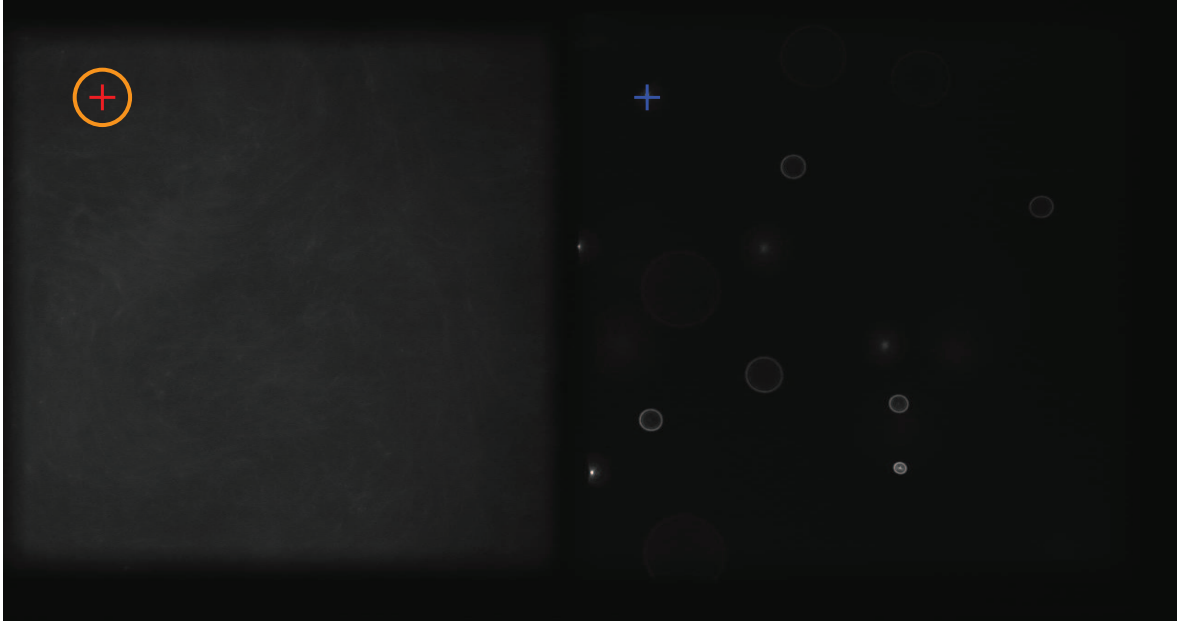


Figure 5.3: A mapping between the location of a detected in-focused particle (blue cross) and its corresponding microtubule bundle (red cross). The small separation distance, Δs , is shown by a circle (yellow).

and neighbor microtubules are nearly the same from different confining geometries. The correlation functions follow the same pattern: decaying to negative values at around $4 \mu\text{m}$ and rising up to zero level as the separation distance, Δs , increasing.

Table 5.1: Revisited data from toroidal channels and cuboid channels.

channel	geometry	coating	cover
toroid (Si II, Chapter 3)	$h = 107 [\mu\text{m}]$	polyacrylamide	glass
toroid (Si III, Chapter 3)	$h = 206 [\mu\text{m}]$	polyacrylamide	glass
toroid (Si IV, Chapter 3)	$h = 306 [\mu\text{m}]$	polyacrylamide	glass
toroid (COC, Chapter 3)	$h = 292 [\mu\text{m}]$	pluronic	COC film
cuboid (Chapter 4)	$H = 0.3 [\text{mm}]$	polyacrylamide	glass
cuboid (Chapter 4)	$H = 0.1 [\text{mm}]$	polyacrylamide	glass

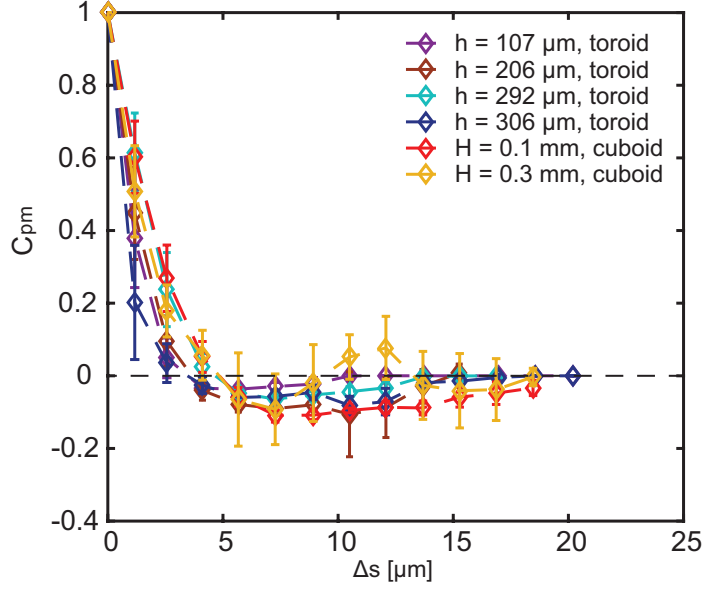


Figure 5.4: Velocity-velocity correlation functions as a function of the separation distance, Δs , from toroidal channels and cubic channels. The height and shape of each channel is denoted in the plot.

5.5 Discussion

Figure 5.4 indicates that the difference in large-scale dynamical states, either coherent or turbulent state, hardly influence the local interaction between active microtubule bundles and passive tracer particles in the active systems. If we estimate the correlation length by integrating positive correlation functions, Figure 5.4 indicates that the length scale of correlated active microtubule bundles and passive tracer particles is less than $2 \mu\text{m}$. Therefore, fluorescent particles well reflect the background flow motion driven by nearby microtubule bundles. The negative correlation indicates the interaction between passive tracer particles and active microtubules sliding in an opposite direction. The appearance of negative correlation at around $4 \mu\text{m}$ implies that the average distance between sliding microtubule fragments is around $4 \mu\text{m}$.

However, there are limitations of our measurement. We are not able to ana-

lyze fluorescent microtubules using the PIV analysis for cuboid channels with larger heights, $H \geq 0.5$ mm, because the background fluorescence in recorded images has an equivalent intensity of in-focus microtubule bundles. The equivalent intensity values between background noises and target bundles significantly influence the cross-correlation analysis (Equation 5.2), therefore, it affects the integrity of displacement analysis. For microtubule visualization in large cuboid channels, we would recommend other microscopic systems, for example, confocal microscopy which blocks out-of-focus wavelengths [92] or light sheet fluorescence microscopy which only illuminates a thin slice of the fluorescent sample [93, 94].

CHAPTER SIX

Conclusion

In this thesis, we presented an experimental set-up based on an epi-fluorescence microscope equipped with a two-color imaging system and a synchronized objective z-drive. The set-up was used to measure the assembled two-color active fluids, which contained red microtubule bundles and blue passive tracer particles. Recorded images of fluorescent particles were analyzed using three-dimensional particle tracking method, which located an individual particle through its Airy disk.

We focused on three-dimensional dynamics of the active fluids confined in a series of toroidal channels. Results show two dynamical states: chaotic turbulent flows in a toroid with a small aspect ratio and large-scale coherent flows in geometries with large aspect ratios. The azimuthal component of velocity in coherent flows (during defined initial time period) increases with the cross section of each confinement, and it is not influenced by the surface treatment of channels. Fluctuations of the active flows — including turbulent and coherent cases — from all axes is dependent on the height, which is the smallest dimension of confining geometry. In contrast, the lifetime and structure of coherent flows are affected by the surface condition. In addition, we find that the azimuthal velocity of large-scale coherence can be scaled by fluctuation.

To study the physical meaning of fluctuation and the basic principle of active flows near confining surfaces, we measure the assembled active systems in a series of cuboid channels with increasing level of one-dimensional confinement. The zero averaged velocity and isotropic velocity fluctuation indicate chaotic turbulent dynamics. In this dynamical state, the activity of the active fluids is reflected by fluctuation. As confining level increases (two planar boundaries approach), the lifetime of activity remains constant, and the isotropic fluctuation strength decreases. The sliding motion of microtubule bundles is restricted by the one-dimensional confinement and leads to the anisotropy at larger scale in the active systems. Large-scale collective

motion is observed with a characteristic size depending on the smallest dimension of each confinement.

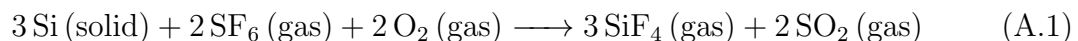
We studied the interaction between active microtubules and passive particles. The interaction shows almost the same velocity-velocity correlation regardless of the dynamical states and confining geometries. The estimated correlation length reveals that an active microtubule bundle affects passive fluorescent particles within less than $2\text{ }\mu\text{m}$ separation distance.

Results presented in this thesis inspire more work. To reveal the surface condition influence, one direction is to study the variation in the dynamics of the coherent flows with the polymer coating concentration. Another direction is to measure two-color active fluids with fluorescence on motor clusters and passive tracer particles, in order to observe the motion of motor cluster and background fluid, which are directly related to the chemical energy consumption and activity in the systems. To further understand the influence of boundaries, one series of measurements are to focus on other locations in one-dimensional confined systems - closer to the walls. A second series of measurements would be to look at the microtubule motion and bundle orientation at different locations in the channel. These, coupled with emerging theories of active systems, will lead to a stronger, more comprehensive understanding of the mechanisms of these innovative active fluids.

APPENDIX A

Channel fabrication

Silicon channel: deep reactive ion etching (DRIE). Deep reactive ion etching (DRIE), compared to traditional RIE, enables high-rate etching of deep and narrow features. It is a process that combines physical etching and chemical etching to remove material from the wafer surface. Ion bombardment enhances wafer surface desorption. Chemical reactions in the vacuum chamber are described as following equations:



In addition to etchant gases, SF_6 and Cl_2 , other gases such as O_2 and C_4F_8 are used to modify plasma chemistry and side wall passivation. Oxygen in SF_6/O_2 mixture controls the amount of free fluorine. C_4F_8 passivates sidewalls in order to minimize lateral etching and produce vertical sidewalls. However, over deposition of polymer may result in residues at trench bottom, which will affect the etched surface quality.

Silicon channels were prepared in an inductively coupled plasma reactive ion etching (RIE) system (SPTS Technologies, Model LPX-ICP) with following procedures:

1. Soft lithography with positive photoresist. Desired toroidal geometry was transferred from transparent mask to silicon wafer surface by positive photoresist (Microchem, S1813, Karl Suss MJB-3 Mask Aligner).
2. Aluminum deposition. For deep etching (generally larger than $50 \mu\text{m}$), a metal mask is favored. A 50 nm aluminum layer was deposited on soft lithography via e-beam evaporation (Lesker, Lab 18).
3. Positive photoresist lift-off. Silicon wafer with photoresist layer and aluminum layer was sonicated in acetone to lift off the photoresist layer and the attached

aluminum layer on top. After lift-off process, silicon surface was exposed.

4. Bosch process [67]. The standard configuration is 600 W ICP power, 20 W CCP power, 130 sccm SF_6 etchant gas flow rate with 7 s etch time and 5 Pa etch pressure, 80 sccm O_2 flow rate, 100 sccm C_4F_8 passivate gas flow rate with 5 s deposition time and 2.5 Pa deposition pressure.

Initially etched silicon wafers were measured by scanning electron microscope (JEOL, JSM 845) to monitor angles of sidewalls (Figure A.1). By increasing process time of each silicon wafer and measuring corresponding feature depth, we rated etching speed for narrow trenches, which was around $2\text{ }\mu\text{m}/\text{min}$. Figure A.2 presents measurements of etched depth in silicon wafers as a function of processing time and a linear estimation of etching speed. We used measured etching speed to calculate etching time of silicon channels and measured the toroidal feature with a profilometer (Zeta Instruments, Zete-20) as shown in Figure A.3.

Cyclic olefin copolymer (COC) channel: CNC-machining. The COC channel was manufactured using a CNC mill machine (MDA Precision LLC., Model V8-TC8 3-axis). The design was sketched in Vectric-Cut2D software and exported to a G-code for CNC-machining. The COC plaque (TOPAS Advanced Polymers, 8007) was fixed on operational stage and the end mill bit (Accupro, $254\text{ }\mu\text{m}$, flat) was calibrated on a flat surface attached to the operational stage for home position. The COC plaque was immersed in water for cooling. During milling, the end tip moved at a slow feed rate and at a low plunge rate, both at $0.1\text{ mm}/\text{second}$. The motor speed was set at 10000 rpm. Manufactured channel was measured with the profilometer as shown in Figure A.4 and Figure A.5.

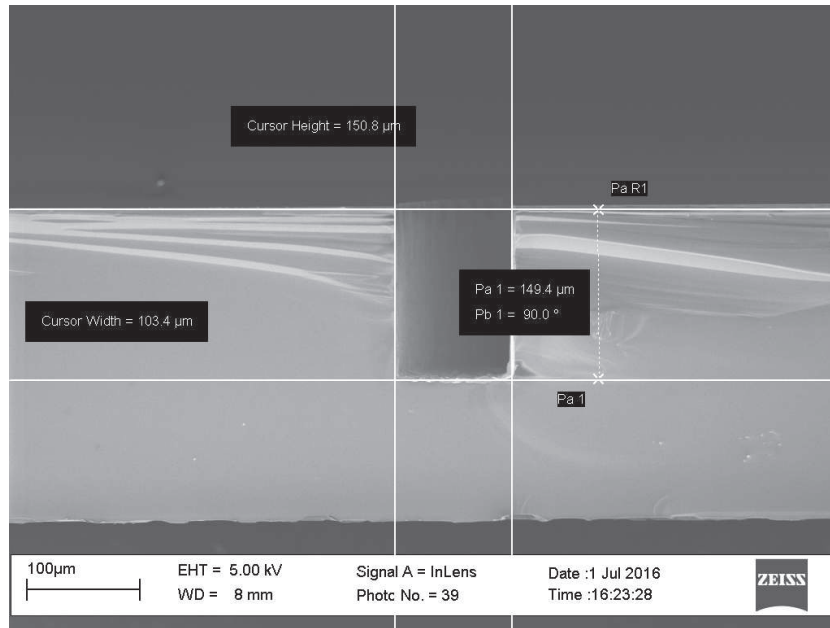


Figure A.1: An SEM image of the cross-section of an etched silicon wafer. Both walls in the toroidal feature show good vertical contour.

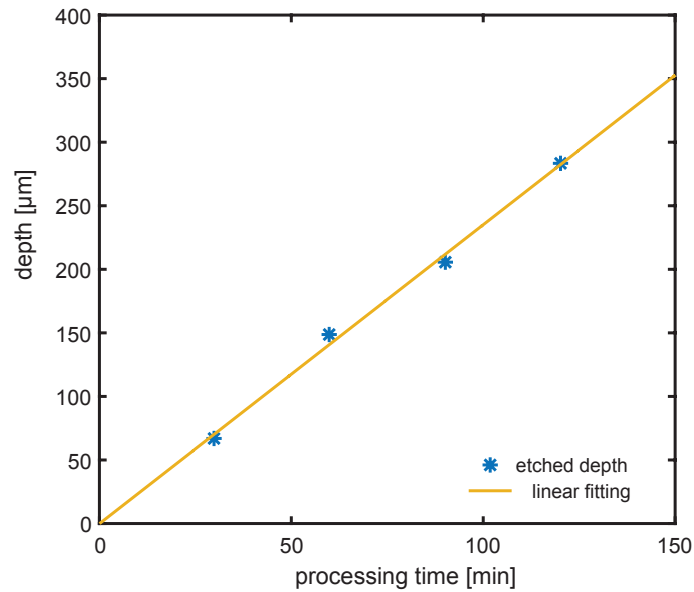


Figure A.2: An SEM image of the cross-section of an etched silicon wafer. Both walls in the toroidal feature show good vertical contour.

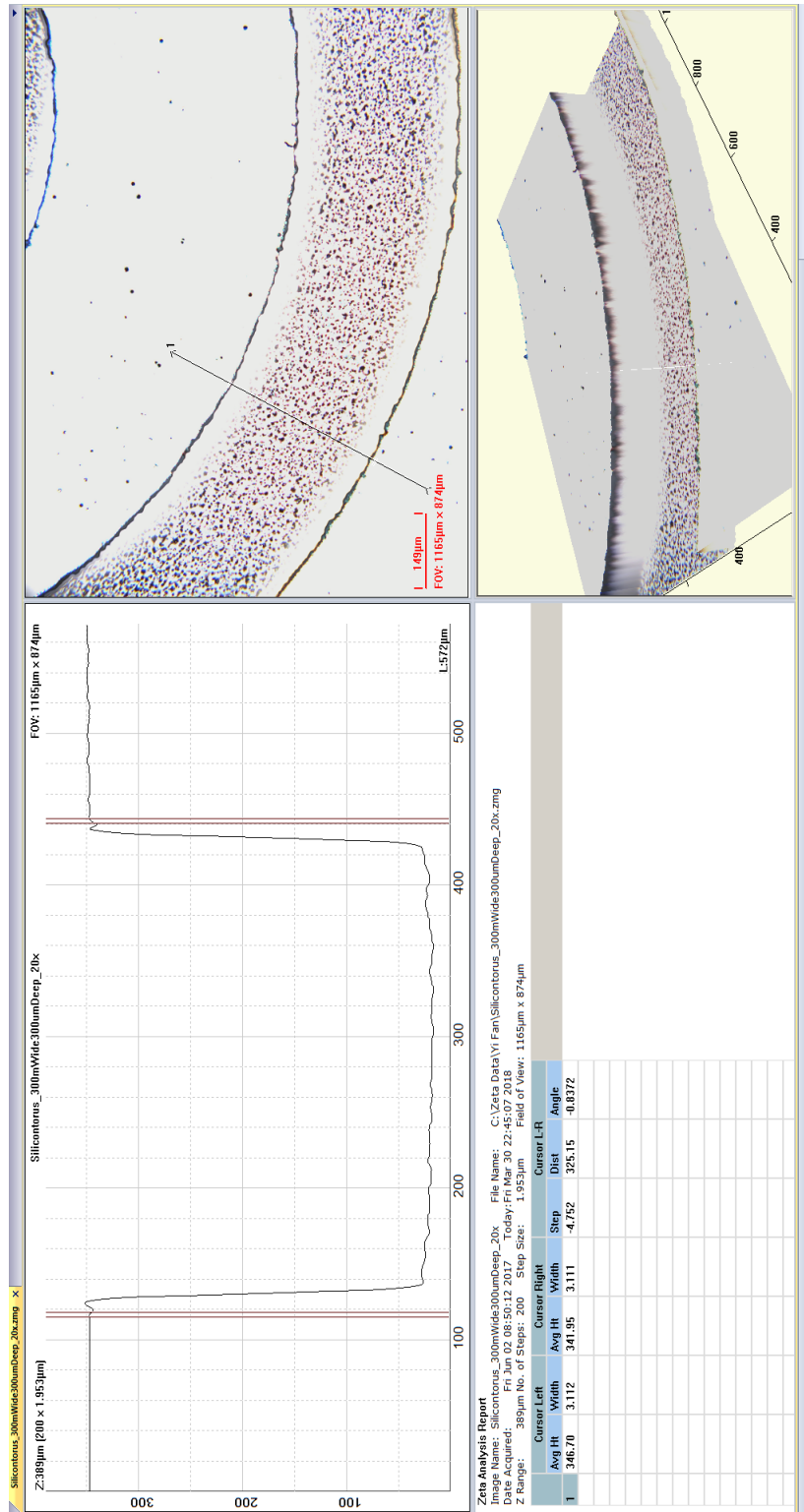


Figure A.3: A profile of the cross-section of an etched silicon wafer in a profilometer.

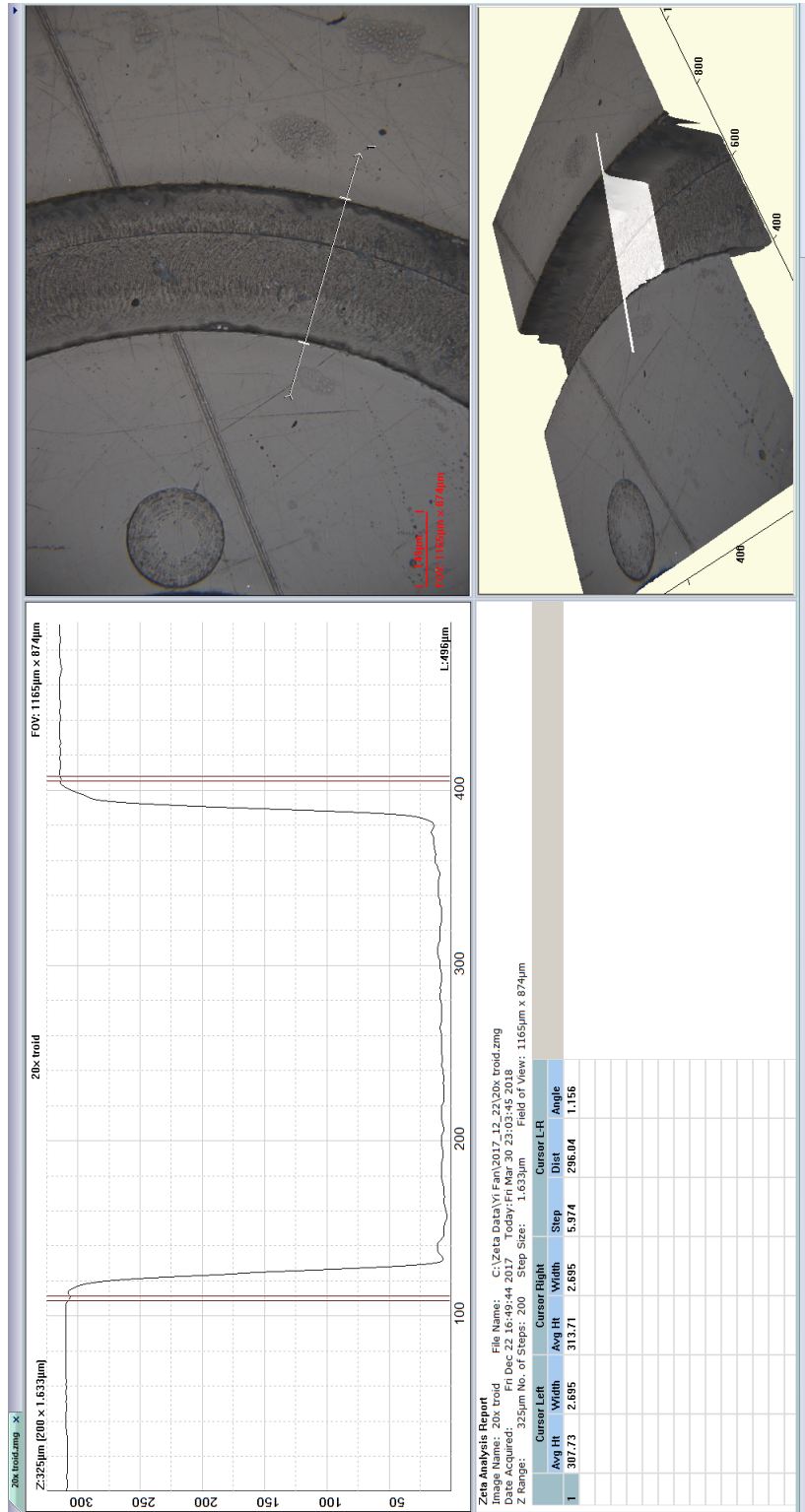


Figure A.4: A profile of a CNC-machined COC toroid in a profilometer.

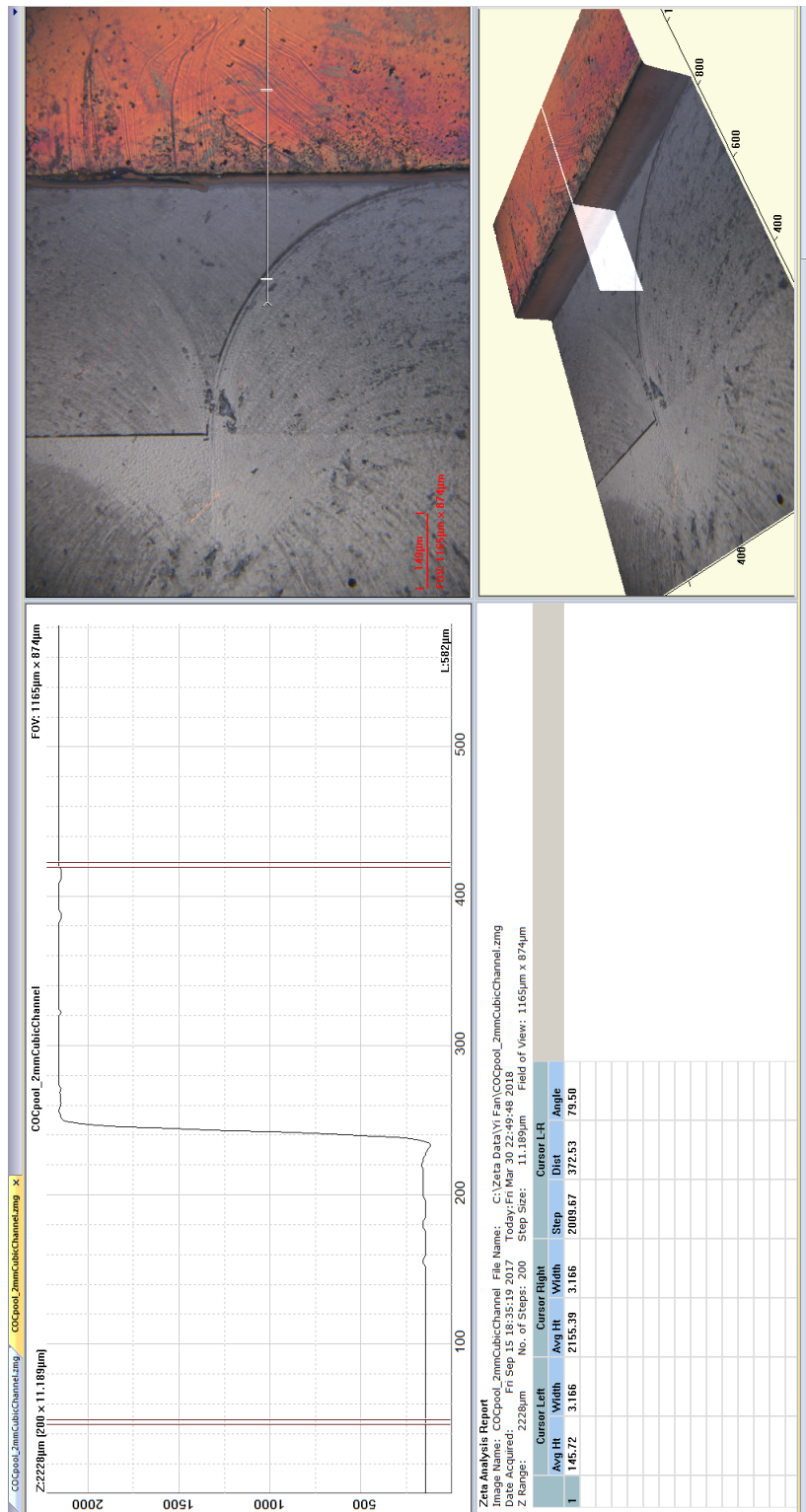


Figure A.5: A profile of a CNC-machined COC cuboid channel in a profilometer.

APPENDIX B

Surface treatment

We followed a standard protocol to treat silicon or glass surfaces, and COC surfaces with poly-acrylamide. The main procedures are:

1. Immerse and sonicate silicon channels for 5 minutes in boiled 1% Hellmanex (volumetric concentration in deionized (DI) water).
2. Rinse sonicated devices thoroughly in DI water and then soak them in ethanol for 10 minutes.
3. Rinse silicon or glass devices thoroughly in DI water and soak them in 100 mM KOH solution for 10 minutes. For COC surfaces, replace 100 mM by 1 M KOH solution for 10 minutes.
4. Prepare silane solution with 100 mL ethanol, 1 mL acetic acid and 0.5 mL trimethoxysilyl-propyl methacrylate ($\text{H}_2\text{C}=\text{C}(\text{CH}_3)\text{CO}_2(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$).
5. Rinse devices thoroughly in DI water and immerse them in silane solution for 10 to 20 minutes.
6. Prepare acrylamide solution. We used 20% acrylamide solution and dilute it to 2% with DI water. Then place 2% acrylamide solution in vacuum chamber to degas for 10 minutes.
7. Take devices out from silane solution, rinse them with DI water and blow dry them with compressed nitrogen (compressed air is fine as well).
8. Polymerize acrylamide. Take acrylamide solution out from the vacuum chamber. Mix 100 mL 2% acrylamide solution with 70 mg ammonium persulfate (APS) and 35 μL Tetramethylethylenediamine (TEMED). Note: let the APS dissolve first while stirring and then add TEMED. Volume and mass can be linearly increased if larger volume of polyacrylamide solution in need.

9. Within 20 seconds after adding TEMED, immerse dried devices into polymerized acrylamide solution. Coating should be ready in ~ 3 hours.

The standard procedure to treat COC surfaces with pluronic to prevent direct contact between proteins and surface is as following:

1. Sonicate COC channels or films in boiled 1% Hellmanex solution for 5 minutes.
2. Immerse COC channels or films in ethanol for 10 minutes.
3. 20 minutes sonication in deionized (DI) water.
4. Dissolve pluronic powder completely in DI water by mixing them for ~ 4 hours to make 2% (w/w) solution.
5. Immerse cleaned COC channels or films in pluronic solution overnight.

APPENDIX C

Assembling active fluids

Tubulin purification, labeling and polymerization.

Raw tubulin was purified from bovine brains through two cycles of polymerization-depolymerization in high-salt 1 M 1,4-piperazinediethanesulphonic (PIPES) buffer at pH 6.8 and stored at -80°C [95]. Then the raw tubulin was polymerized and depolymerized for a third time and flash-frozen by liquid nitrogen at a high concentration (usually larger than 20 mg/mL, 44.9 mg/mL in this case) using thin-walled tubes. For fluorescence microscopy, a portion of recycled tubulin at the high concentration was labeled with Alexa Fluor 568 (Thermal Fisher Scientific, A20003) by a succinimidyl ester linker. Spectrum absorbance indicated that 34% of tubulin monomers were labeled. To prepare microtubule for the active fluids mixture, recycled tubulins (not fluorescent) were co-polymerized with Alexa 568 labeled tubulins at 37°C for 30 min to produce 3%-labeled microtubules. To promote and stabilize polymerization of microtubules, 600 μM GMPCPP (guanosine-5'-[(α,β)-methylene]triphosphate (Jena Biosciences, NU-4056), 1 mM dithiothreitol (DTT) and M2B buffer were added. The target polymerization concentration was 8 mg/mL, which can affect polymerization speed and the final length of microtubule filaments. The resulting length has an average value of $\sim 1\ \mu\text{m}$ [27, 28]. Microtubules were stored in small aliquots of 10 μL at -80°C .

Kinesin-streptavidin complexes.

We used K401 derived from *Drosophila melanogaster* kinesin, which consists of 401 amino acids of the N-terminal motor domain with a biotin tag [96]. The biotin tag helped to assemble kinesin motors to multi-motor clusters using streptavidin tetramers (Invitrogen, S-888). Linking kinesins into motor clusters permits binding among multiple microtubule filaments and introduces inter-filament sliding. The mixing ratio of kinesin motors (1.5 μM) and streptavidin (1.8 μM) was 1 : 1.2 in

M2B buffer (M2B: 80 mM PIPES, 1 mM EGTA, 2 mM MgCl_2 , pH 6.8). 120 μM DTT was added before 30 min incubation at 4°C. After preparation, the kinesin-
straptavidin mixture was stored in aliquots at -80°C .

Kinesin-driven active fluids.

Microtubules and motor clusters were prepared separately and combined with other mixtures in a high-salt buffer (M2B + 3.9 mM MgCl_2). We added an ATP regeneration system, as the most important energy supply for motor clusters, using 26 mM phosphoenolpyruvate (PEP) and 2.8% (v/v) stock pyruvate kinase/lactic dehydrogenase enzymes (PK/LDH) (Sigma, P0294). Motor clusters hydrolyze ATP to ADP while stepping on microtubule filaments. PK consumes PEP and converts ADP back to ATP, therefore, the ATP concentration in the active systems remain stable until PEP and ATP are depleted. To reduce photobleaching effect, we minimized the oxygen exposure of fluorescent samples by adding 2 mM trolox (Sigma, 238813) and anti-oxidants [97], including 0.22 mg/mL glucose oxidase(Sigma, G2133), 0.038 mg/mL catalase (Sigma, C40) and 3.3 mg/mL glucose. We added 5.5 mM DTT to stabilize proteins, 2% (w/w) Pluronic F127 to encourage bundling of microtubule filaments and $\sim 0.001\%$ (v/v) fluorescent particles to probe the active systems. The final concentration of microtubules is 1.3 mg/mL, kinesin is 1.5 μM and ATP is 1.4 mM.

APPENDIX D

Validation

To validate the three-dimensional tracking velocimetry, we recorded a collection of fluorescent particles diffusing in deionized (DI) water, (i.e. Brownian motion). The out-of-focus particles were tracked from frame to frame in three-dimensions based on their Airy disks. Each image corresponds to a time instant, t , and each particle in that image, denoted by the index j ($j = 1, 2, 3, \dots$), has a position, $\mathbf{X}^j$ (in a Cartesian coordinate system):

$$\mathbf{X}^j(t) = (x^j(t), y^j(t), z^j(t)). \quad (\text{D.1})$$

From this time history of particle positions, we can compute the mean square displacement (MSD) as:

$$\begin{aligned} \langle d_x^2(\Delta t) \rangle &= \frac{1}{P} \sum_{j=1}^P (x^j(t + \Delta t) - x^j(t))^2, \\ \langle d_y^2(\Delta t) \rangle &= \frac{1}{P} \sum_{j=1}^P (y^j(t + \Delta t) - y^j(t))^2, \\ \langle d_z^2(\Delta t) \rangle &= \frac{1}{P} \sum_{j=1}^P (z^j(t + \Delta t) - z^j(t))^2. \end{aligned} \quad (\text{D.2})$$

Here, the angle brackets, $\langle \dots \rangle$, denote an ensemble average over all particles, P , collected from all image frames. Figure D.1A shows the trajectory of a typical particle experiencing Brownian motion. Figure D.1B shows mean square displacements (Equation D.2), MSDs, based on the total number of traced particles as a function of time lag, Δt . Measured MSDs were fitted by linear functions to determine the slopes in three axes: $l_x = 0.99$, $l_y = 0.97$, $l_z = 0.99$. The results were compared with the theoretical prediction using the Stokes-Einstein equation [98]:

$$D = \frac{k_B T_o}{6\pi\mu a}, \quad (\text{D.3})$$

where k_B is the Boltzmann constant, T_o is the absolute temperature, μ is water dynamic viscosity at the absolute temperature and a is tracer particle radius. From Equation D.3, we find that the theoretical prediction about the slope of MSD is $l = 2D = 0.98$, identical for each axis of x , y and z . The discrepancy between the experimental measurement and the theoretical prediction is 1.0%.

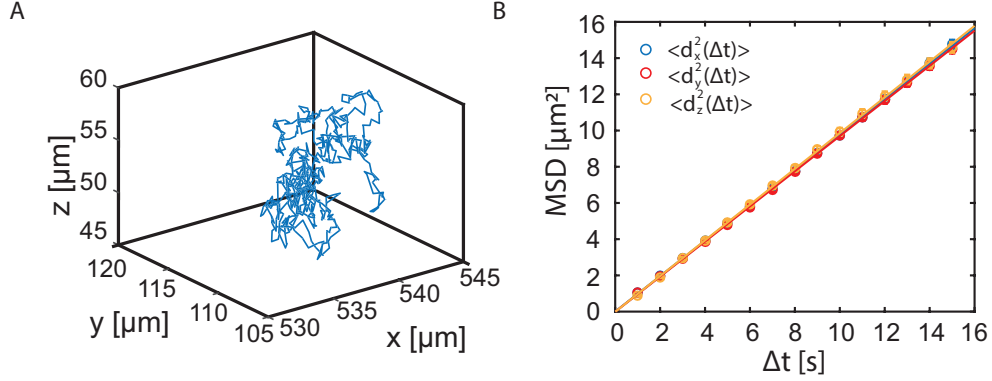


Figure D.1: (A) A trajectory of one particle performing Brownian motion in DI water at room temperature. (B) Mean square displacement of particles (circle) and corresponding linear fitting function (solid line) in the Cartesian coordinate system.

To validate with different particles and different channels used in Chapter 4, we recorded a collection of fluorescent particles (1 μm diameter or 2.9 μm diameter) diffusing in deionized (DI) water confined in three geometries of COC channels, $H = 4 \text{ mm}$, $H = 2 \text{ mm}$ and $H = 0.1 \text{ mm}$. The out-of-focus particles were tracked from frame to frame in three-dimensions based on their Airy disks. Each image corresponds to a time instant, t , and each particle in that image, denoted by the index j ($j = 1, 2, 3, \dots$), has a position, $\mathbf{X}^j$ (in a Cartesian coordinate system):

$$\mathbf{X}^j(t) = (x^j(t), y^j(t), z^j(t)). \quad (\text{D.4})$$

From this time history of particle positions, we compute mean square displacement

as:

$$\langle d^2(\Delta t) \rangle = \frac{1}{P} \sum_{j=1}^P \left((x^j(t+\Delta t) - x^j(t))^2 + (y^j(t+\Delta t) - y^j(t))^2 + (z^j(t+\Delta t) - z^j(t))^2 \right). \quad (\text{D.5})$$

The angle brackets, $\langle \dots \rangle$, denote an ensemble average over all particles, P , collected from all image frames in the validation data set.

Figure D.2 shows mean square displacements (MSDs) of Brownian motion at central levels in three geometries. MSDs (Equation D.5) are averaged on the total number of traced particles and normalized by the size of particles, 1 μm diameter or 2.9 μm diameter, as a function of time lag, Δt . In a log-log plot, MSDs are linear functions of tracking time and error bars indicate standard errors of the averaging over traced particles. In the 2 mm cubic channel and the $2 \times 2 \times 0.1 \text{ mm}^3$ channel, MSDs are calculated based on 3D particle tracking with 1 μm diameter particles; and for 4 mm cubic channel, MSD is from 2D tracking with 2.9 μm diameter particles. We fit linear functions to MSDs and the corresponding slopes are $l_1 = 1.03$, $l_2 = 1.00$ and $l_3 = 1.07$ (0.37 if not normalized by the size of particles). Theoretical diffusion coefficient, D , can be derived from Stokes-Einstein equation [98] (Equation D.3). For 1 μm particle, $D = 0.49$, as a consequence, the slope of MSD from theoretical prediction is $2D = 0.98$ (2.9 μm particle, $2D_3 = 0.34$ if not normalized). For 1 μm particles, the discrepancy between experimental results and theoretical prediction is 2.0% in channel $H = 0.1 \text{ mm}$, and 5.1% in channel $H = 2 \text{ mm}$. With 2.9 μm particles, it increases to 8.8% because we obtain lower signal-to-noise ratio in fluorescent images when focus at longer distance, $\Delta z = 2 \text{ mm}$ in channel $H = 4 \text{ mm}$. Therefore, our system shows 94.9% accuracy for detecting displacement less than 0.7 μm (calculated from 1 μm particles in 2 mm channel) and 91.2% accuracy for detecting displacements less than 0.4 μm (calculated from 2.9 μm particles in 4 mm channel). MSDs from

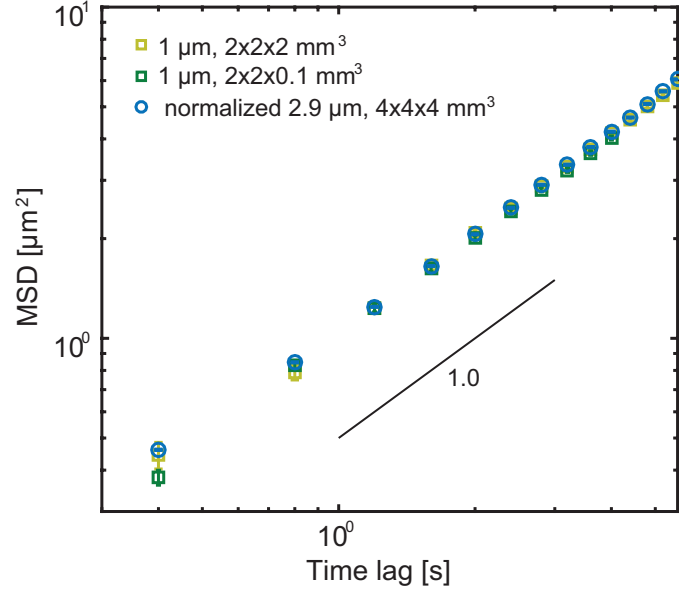


Figure D.2: Mean square displacements of $1\ \mu\text{m}$ and $2.9\ \mu\text{m}$ fluorescent particles in deionized water confined in three channels. Error bars indicate standard errors of mean square displacements among total number of traced particles. Solid line is exponent of 1.

Figure D.2 also reveal that diffusive motions of particles in center volumes from difference channels are not affected by boundary surfaces. Constrained Brownian motion of isolated spherical particle is highly dependent on the ratio, l/a , between the distance from the particle center to confining surface, l , and the particle radius a [99]. The wall drag effects arise when the ratio satisfies $l/a < 4$ and lead to hindered diffusion of isolated particles [80, 81]. In our measurements, the gap-size-to-radius ratio, l/a , is much larger, therefore, the influence on particles from walls are negligible.

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