<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Harnessing fluorescent nano-material to visualize microfluidics (Main article)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Xiao, Lian</td>
</tr>
<tr>
<td><strong>Citation</strong></td>
<td>Xiao, L. (2018). Harnessing fluorescent nano-material to visualize microfluidics. Doctoral thesis, Nanyang Technological University, Singapore.</td>
</tr>
<tr>
<td><strong>Date</strong></td>
<td>2018-12-31</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10220/47311">http://hdl.handle.net/10220/47311</a></td>
</tr>
<tr>
<td><strong>Rights</strong></td>
<td></td>
</tr>
</tbody>
</table>
Harnessing Fluorescent Nano-Material to Visualize Microfluidics

Xiao Lian

2018
Harnessing Fluorescent Nano-Material to Visualize Microfluidics

Xiao Lian

School of Physical and Mathematical Sciences

A thesis submitted to the Nanyang Technological University in fulfilment of the requirement for the degree of Doctor of Philosophy

2018
Acknowledgments

During the past 4 years at Nanyang Technological University, I have received helps and hindrances from various persons around me, while both of them accelerate my personal growth, thus I really appreciate them.

Firstly and foremost, I would like to express my sincere appreciation to the MOU signed between the Schools of Sichuan University and Nanyang Technological University, which offers me the opportunity to pursue my Ph.D. degree at Nanyang Technological University. And it is with immense gratitude that I acknowledge the support and help of my supervisor Associate Professor Sun Handong, I would never be able to pass my QE and finish my Ph.D. thesis without his kind help and support. I would like to show my deepest gratitude to Professor Sun Handong for providing me freedom and patience, thus I can study independently and touch different research fields. I also thank Associate Professor Wong Teck Neng for his kind support.

I greatly appreciate Dr. Huang Yi for his valuable suggestions and help in both research and daily life.

I would like to appreciate the help from Dr. Wang Yue, Dr. Zhao Xin, Dr. Yang Shancheng, Dr. Wang Zeng, especially at the first 2 years of my PhD study. I also thank them for sharing some of their experimental skills.

I would like to appreciate the following group members and friends, Dr. Xu Hongyi, Dr. Gao Yuan. Dr. Wang Lin, Dr. Gu Zhiyuan, Dr. Zhou Xinxing, Mr. Lim Wenxiang, Ms. Chen Xiaoxuan, Mr. Cheng Shijia, Mr. Gong Chen.

I also would like to acknowledge the Research Scholarship provided by Nanyang Technology University.
Table of Contents

Acknowledgments .............................................................................................................. i

Table of Contents ............................................................................................................ ii

List of figures .................................................................................................................... vi

Abstract ............................................................................................................................... xiii

List of publications ............................................................................................................ xvi

Citations to published work .............................................................................................. xvii

Chapter 1. Introduction ...................................................................................................... 1

1.1 Fluid theory .................................................................................................................. 3

1.2 Conservation laws ........................................................................................................ 7

1.2.1 Continuity equation ................................................................................................. 7

1.2.2 Momentum conservation equations ......................................................................... 8

1.3 Boundary condition ..................................................................................................... 12

1.4 Microfluidics: low Reynolds number flow ................................................................ 15

1.4.1 Immiscible fluids parallel laminar flows: vertical pinned interfaces ................. 18

1.4.2 Mixing problems at micro-scale and Pe number ..................................................... 21

1.4.3 Droplet and Ca number ........................................................................................... 29

1.5 Significance and motivations of this study: Explore new fluorescent nano materials to
visualize microfluidics ........................................................................................................ 33

1.5.1 Bio-microfluidic visualization: Carbon dots ........................................................... 34
1.5.2 High speed flow field measurement: CsPbBr3 quantum dots and self-assembled CsPbBr3 micro seeding particles

Chapter 2. Experimental Techniques

2.1 Sample synthesis

2.1.1 Carbon dots synthesis

2.2.2 C-dots and C-dots micro particles synthesized for microfluidic visualization

2.2.3 Synthesis of the CsPbBr3 perovskite QDs

2.3 Steady state PL measurement

2.4 Time resolved PL

2.5 Home-made microfluidics fluorescent imaging system

2.6 Microchip Fabrication methods

Chapter 3. Carbon dots emission mechanism: self-trapped exciton

3.1 Introduction

3.2 Results and discussion

3.3 Conclusion

Chapter 4. Dynamic Visualizations in Microfluidics Enabled by Fluorescent Carbon dots

4.1 Introduction

4.2 Results

4.2.1 Optical characteristics of C-dots

4.2.2 Visualizations of mixing via electrohydrodynamic instability
4.2.3 Visualizations of Interface cladding and control of interfacial positions

4.2.4 Visualizations of Droplets and complex droplets

4.2.5 Flow field tracking by fluorescent C-dots micro particles (CDsP)

4.3 Discussion

4.4 Conclusions

4.5 Experiments and simulations:

4.5.1 Chemical inertness of C-dots

4.5.2 Photostability of C-dots

4.5.3 CCK-8 assay for the cytotoxicity

4.5.4 Fluidic sample prepare for the experiments

4.5.5 Microchannels utilized in the experiments

4.5.6 Numerical simulations on droplet formations

Chapter 5. Enabling Quantitative Investigation of Fast and Complex Flow Field in Microfluidics by Self-assembled CsPbBr$_3$ Perovskite Flow Tracers

5.1 Introduction

5.2 Results

5.2.1 Self-assembling and characterizations of the perovskite QDs based particles (PQDsP)

5.2.2 Micro-flow measurements in laminar flow systems

5.2.3 Micro-flow measurement in droplets

5.2.4 Applications of PQDsP in complex emulsion systems
5.3 Discussion and summary ................................................................. 105
5.4 Materials, methods and simulations ............................................. 108
  5.4.1 Microchip fabrications .............................................................. 108
  5.4.2 Fluidic sample preparation for the experiments ......................... 110
  5.4.3 Interfacial tension characterizations ......................................... 111
  5.4.4 Microfluidic configurations ...................................................... 111
  5.4.5 Computational fluid dynamics (CFD) simulations—the model of velocity field and interface tracking ......................................................... 111

Chapter 6. Summary and Prospect ....................................................... 116

6.1 Summary .......................................................................................... 116
  6.1.1 Carbon dots emission originate from self-trapped exciton .......... 116
  6.1.2 Dynamic Visualizations in Microfluidics Enabled by Fluorescent Carbon Nanodots ............................................................................. 117
  6.1.3 Quantitative Investigation of Fast and Complex Flow Field in Microfluidics: Self-assembled CsPbBr3 Perovskite Flow Tracers ......................... 117

6.2 Outlooks ......................................................................................... 118

References ............................................................................................ 119
List of figures

Figure 1.1 Three kinds of fluid flows. (a) extensional flow, (b) shear flow, (c) rotation flow

Figure 1.2 Surface forces along x direction

Figure 1.3 Normal velocity boundary condition

Figure 1.4 The vertical wall generated by two immiscible flows

Figure 1.5 Various applications based on immiscible parallel laminar flows

Figure 1.6 Species fluxes for a Cartesian control volume

Figure 1.7 Stretching and folding process can decrease the effective diffusion length

Figure 1.8 Micro channel for the mixing to happen

Figure 1.9 Applications of miscible parallel laminar flows. (a) T sensor, (b) H filter, (c) Motile sperm extraction

Figure 1.10 Monodispersed droplet generated by T junction (a) and flow focusing (b)

Figure 2.1 Imagines of carbon dots samples. (a) carbon dots sample A, (b) carbon dots sample B dissolved in 150 ml DI water, (c) carbon dots sample B dissolved in 45 ml DI water, (d) carbon dots sample B based seeding particles. (a), (b) are the carbon dots samples for the optical spectroscopy characterization, and the (c), (d) are the carbon dots samples for the microfluidic applications

Figure 2.2 Set-up for steady states PL measurement

Figure 2.3 Set-up for polarized PL measurement

Figure 2.4 Time-resolved PL measurement by streak camera

Figure 2.5 Home-made microfluidic fluorescent imaging system

Figure 2.6 Microchip fabrication process

Figure 3.1 XRD spectra of carbon dots. (a), (b) for sample A and B respectively

Figure 3.2 Raman spectra of carbon dots. (a), (b) for sample A and B respectively
Figure 3.3 (a) Absorption and PL (excited by 442 nm) spectra of sample A. (b) Absorption and PL (excited by 442 nm) spectra of sample B. .................................................................50

Figure 3.4 Emission anisotropy and degree of linear polarization of film Sample A (figure (a), (c)) and film sample B (figure (b), (d)). ............................................................................................................51

Figure 3.5 (a), (c) Excitation anisotropy and degree of linear polarization for sample A, (b), (d) are the results for sample B. Both the excitation anisotropy and polarization show similar behaviors of emission. Excitation wavelength: 442 nm. ......................................................52

Figure 3.6 Emission anisotropy and degree of linear polarization of solution Sample A (figure (a), (c)) and solution sample B (figure (b), (d)). Excitation light: 442 nm. .................................................................55

Figure 3.7 (a),(c) Excitation anisotropy and degree of linear polarization for sample A aqueous solution, (b),(d) are the results for sample B aqueous solution. Both the excitation anisotropy and polarization show similar behaviors of emission. Excitation wavelength: 442 nm........56

Figure 3.8 Electric-field modulation of sample A (figure (a)) and sample B (figure (b)). Insert is the emission spectra of C-dots sample. Excitation light: 442 nm...............................................57

Figure 3.9 Power dependent PL of sample A (figure (a), (c)) and sample B (figure (b), (d)). The unit of excitation power in figure (a) and (b) is mW/cm². Excitation light: 442 nm.......59

Figure 3.10 (a) Time-resolved spectroscopy measurements of film Sample A. (b) Time-resolved spectroscopy measurements of film sample B. .................................................................60

Figure 3.11 Time-resolved results for the C-dots aqueous solution. (a),(b) for sample A and B aqueous solution respectively. .................................................................................................61

Figure 3.12 Fitting results based on the one-phonon emission approximation model. ........63

Figure 3.13 Electronic structure of C-dots (figure (a)). The excitation dependent photoluminescence of sample A (figure (b)) and sample B (figure (c)). Polarization spectra excited by different light, sample A (figure (d)) sample B (figure (e)), blue line: excited by 325 nm, green line: excited by 442 nm. .................................................................................................64
Figure 3.14  Structure of the glucose (figure (a)) and the possible self-trapped exciton structure: figure (b) (C-O bond rupture) and figure (c) (peroxy radical bond: the O atom insert into the C-O bond). Red: O, white: H, grey: C .................................................................66

Figure 4.1 (a). TEM image of the synthesized C-dots. (b) Excitation dependent PL of C-dots ..................................................................................................................................................69

Figure 4.2 Optical properties of C-dots in DI water. (a) Spectra of photoluminescence (PL) and absorption of the C-dots, (b) C-dots sample excited by a 442 nm laser. .................................70

Figure 4.3 (a), (b) Chemical inertness of the synthesized C-dots solution. (a) PL of the C-dots solution, (b) image of C-dots solution excited by a 442 nm laser. The C-dots sample was synthesized on 07 December, 2015 and the test was carried out on 07 December, 2016. (c) Photostability of the C-dots solution by plotting the integrated intensity of the PL spectra for 3 hours with the frequency of 6 times per hour. .........................................................................................71

Figure 4.4 Cell survivability of 293T and HeLa after culturing with C-dots for 24 hours. The measurement was carried out by using of CCK-8 assay.................................................................72

Figure 4.5 Electrohydrodynamic instability induced interfacial mixing characterized by C-dots. (a) schematics of the proposed interfacial mixing method, (b) mixing evolution at applied electric field strength of 136.36 KV/m, (c) mixing procedure characterized by mixing efficiency, (b) maximum mixing efficiency achieved by various electric field strengths. Scale bars represent 100 µm. The electric field is applied by inserting two Pt wires into the inlet of C-dots and the outlet acting as the anode and cathode respectively. ........................................74

Figure 4.6 Flow switching by a core-cladding configuration. (a) Schemes of the core-cladding configuration, (b) positions of the core phase (C-dots) under various flow rate ratios ($\frac{Q_{l1}}{Q_{l2}}$), (c) positional dependency of the core phase on the flow rate ratio between the two cladding phases, where the location was non dimensionalized by the width of the microchannel. Scalar bars indicate 50 µm.................................................................76
Figure 4.7 Applications of C-dots in droplets and complex droplets. (a) Droplet formations in three typical regimes: squeezing, dripping and jetting, (b) correlations between capillary number and droplet diameter. Scalar bars represent 50 µm. ........................................78

Figure 4.8 (a) Various double component droplets with C-dots and mixture of glycerol & DI water (gly. mix.) as the dispersed phase (b) merging of such double components droplet under a normally applied DC electric field at the strength of 9 MV/m. Scalar bars represent 50 µm. ..................................................................................80

Figure 4.9 (a) Schematics of double emulsion formation with C-dots in outer droplets, (b) formation dynamics of double emulsions. Scalar bars represent 50 µm. .................................82

Figure 4.10 Flow field measurement in microfluidics by employing CDsPs. (a) SEM image of the synthesis CDs, (b) PL spectra of the porous polystyrene micro particles before and after synthesis, (c) the fluorescent image of droplet formation with CDsps distributed in dispersed phase, (d) typical flow field of droplet formation obtained by micro-PIV correlation of the recorded imaged illuminated by CDsps, (e) simulated flow fields of droplet formation. Scale bars represent 50 µm if not specifically indicated. Both the flow measurement and simulation were carried out at the flow rates of CDsps aqueous solution versus mineral oil based solution =20 : 100 µL/h. .........................................................................................84

Figure 4.11 Schematics of the microchannels employed for interfaces formed by two types of liquid phases. (a) The microchannel used for mixing via electrohydrodynamic instability (figure 4.5), (b) microchannel utilized for interface cladding (figure 4.6). .........................88

Figure 4.12 Microchannel utilized for droplet generations and flow field measurement. (a) Schematics of the microchannel for droplet formation and double component droplets, (b) photo of the corresponding microchannel, (c) schematics of the microchannel for generation of double emulsions, (d) photo of the microchannel for formation of double emulsions, (e)
schematics of the microchannel for flow field measurement via utilizing CDsPs. The red ink filled into the microchannels in (b) (d) was for better illustrations.

**Figure 4.13** Mesh and phase plot of the simulation for droplet formations. (a) Overall view of the generated mesh, (b) enlarged view of the mesh at the junction, (c) phase plot during the droplet formation process.

**Figure 5.1** Schematics and characterizations of the synthesized perovskite QDs based particles (PQDsPs). (a) Synthesized CsPbBr$_3$ QDs in Hexane, (b) stir-mixing of CsPbBr$_3$ QDs in Hexane solution and mineral oil with 0.2% span 80, (c) Synthesized PQDsPs in mineral oil, (d) TEM image of the CsPbBr$_3$ QDs, (e) Size distribution PQDsP, (f) photo of the PQDsPs in mineral oil with 0.2% span 80, (g) PL spectra of the synthesized PQDsPs dispersed in mineral oil with 0.2% span 80, (h) & (i) are the images of PQDsPs under normal bright field microscopy and fluorescent microscopy configurations respectively. Scale bars in (h) (i) represent 20 µm.

**Figure 5.2** PQDsP enabled quantitative measurement on laminar flow systems. (a) Fluorescent microscopy view of PQDsPs flowing in microchips, (b) vector field of the single-layer laminar flow, (c) flow profiles obtained from the measurement and CFD simulations Scale bars in (d) equal 50 µm.

**Figure 5.3** (a) particle trace images of three-layer flow system under various flow ratios, (b) vector field of the three-layer flow system, The samples indicated in (a) are glycerol 65% in D.I. versus PQDsP in mineral oil with 0.2% span 80 versus glycerol 65% in D.I. Details can be seen in Chapter 5.4.2, Table 2. Scale bars in (b) equal 50 µm.

**Figure 5.4** (a) Velocity distributions of the laminar flow systems (figure 5.3 (a)) via PQDsP enabled quantitative measurement, (b) comparison of the interfacial positions between the experiments and simulations with a constant $Q_c = 50 \mu$L/h.

**Figure 5.5** Droplet formations in T-junction geometry on different flow rates. (a), Droplet formation under the flow rate ratio of $Q_c:Q_s=5:200 \mu$L/h ; (b), (c) Droplet formation under the
flow rate ratio of $Q_d:Q_a=5:100 \mu L/h$; (d) ~ (e) Droplet formation under the flow rate ratio of $Q_d:Q_a=10:100 \mu L/h$. $Q_d$ and $Q_a$ illustrate the flow rate of the dispersed phase and the carrier phase respectively.

**Figure 5.6** Ultra-fast flow measurement of droplets in microchips enabled by the PQDsP. (a) Fluorescent image of droplet generated in T-junction geometry, where PQDsPs are placed in carrier phase and the flow rate ratio is set to be $Q_d:Q_a=5:200 \mu L/h$, (b) flow field of the carrier phase, (c) & (d) enlarged view of the flow field at two critical positions.

**Figure 5.7** (a) fluorescent image of the droplet in microchip with PQDsP in dispersed phase and the flow rate ratio being $Q_d:Q_a=25:200 \mu L/h$, (b) flow field of the droplet indicated in (a), (c) velocity field after subtraction of the mean velocity to reveal the key flow patterns and the enlarged view of the flow recirculation patterns, (d) simulated result of the corresponding flow field in the droplet for comparison. $Q_d$ and $Q_a$ stand for the flow rate of the dispersed phase and the carrier phase respectively.

**Figure 5.8** Ultra-fast flow field measurement of the complex droplets (double emulsions). (a) Fluorescent image of the double emulsion with a single core, (b) the corresponding flow field of the droplet in (a); (c) & (d) are the fluorescent image and flow field of the double emulsion with double core respectively, (e) & (f) are the results of the double emulsion with tribble cores, (g) ~ (i) are the flow recirculation patterns within the tribble-core double emulsion revealed by subtraction of the mean velocity. The materials used for generation of the double emulsions are DI water with 2% (w.t.) Tween 20 as the inner or core droplet ($Q_{core-drop.}$), PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80 as the outer droplet ($Q_{out-drop.}$), 70% (w.t.) glycerol in DI water with 0.3% (w.t) SDS as the outer carrier phase ($Q_{Ca}$). The flow rate ratios for generation of the double emulsions are $Q_{core-drop.}:Q_{out-drop.}:Q_{Ca}=10.5:22:350 \mu L/h$ for one core, $Q_{core-drop.}:Q_{out-drop.}:Q_{Ca}=12:25:350 \mu L/h$ for two cores, $Q_{core-drop.}:Q_{out-drop.}:Q_{Ca}=12:25:250 \mu L/h$ for three cores respectively.
Figure 5.9 Schematics of the microchip designs adopted in the experiments. (a) Microchip utilized in single layer laminar flow, (b) microchip design for the three-layer flow experiments, (c) geometries for the droplet generation in T-junction, (d) microchip design for droplet formation in flow focusing configuration, (e) microchip employed for formation of complex droplets (double emulsions).

Figure 5.10 Uniform mesh used in the calculation.

Figure 5.11 The velocity distributions.

Figure 5.12 Interface distributions at different flow rate ratios.
Abstract

Microfluidics have been considered as a remarkable platform to explore fluid dynamics at micro scale as well as the versatile applications. Fluid flow visualization is the prerequisite of investigating a microfluidic system regarding both fundamental fluid mechanics and practical applications, but currently utilized fluorescent materials cannot meet the requirement for microfluidic visualization, which extremely obstructs their widespread practical applications. This thesis aims to address the issues relevant to microfluidic visualization. The major results are summarized as below.

For biologic related microfluidic studies, such as bio-system mimic, pharmaceutical synthesis, drug delivery etc., the fluorescent tracer must have the combined characters of photo stability, chemical inertness, bio-compatibility, and mostly, low cost. Carbon dots fulfill all the requirements of microfluidic bio-applications, thus have been considered to be applicable for the fluid flow visualization. However, the contentious emission mechanism of carbon dots hinders the applications of these materials. Therefore, we first investigate systematically the emission mechanism of carbon dots by employing polarization anisotropy spectroscopy, electric-field modulation spectroscopy, and time-resolved photoluminescence (PL) measurements. Our results offered strong evidences that carbon dot emission originates from the self-trapped excitons, where the mobilization of the hot carriers is substantially obstructed due to the presence of a strong local potential field and thus the relaxation and decay process of the hot carriers are largely quelled. Our exploration furnishes an insight into the emission mechanism of carbon dots, which enhances our awareness of these novel materials.

The understanding of carbon dots emission allows us to apply these novel materials into microfluidic visualization. For the first time, we document fluorescent carbon dots as a game-changer, applicable in versatile fluidic environment for the visualization in microfluidics with unprecedented advantages, such as photostability, chemical inertness, relative high imaging
intensity, biocompatibility, environmental friendliness and above all, low cost. We have achieved a high fluorescent imaging speed up to 2500 frames per second under a normal continuous wave (CW) laser by utilizing carbon dots in microfluidics. In addition to the interface visualization, carbon dots based micro-particles (also called seeding particles), which enable quantitative investigation of bio-fluidic dynamics at micro-scale with a substantially lower cost, which is inaccessible by traditionally adopted fluorescent dye based seeding particles. Our findings hold profound influences to microfluidic investigations and may even lead to revolutionary changes to the relevant industries.

The high speed dynamics at micro scale also finds various applications such as high efficiency sorting, chemical reaction, fast and high efficient mixing etc. Consequently, high-speed capability is highly demanded for micro particle image velocimetry (µPIV). To elevate the speed, current solutions dominantly focus on the illumination sources---more powerful lasers. Yet, the achievable speed is still insufficient to capture fast and complex flow fields. Hence, we have conceptualized and proposed an alternative solution of achieving ultra-fast quantitative measurement at micro-scale by self-assembling CsPbBr3 quantum dots into fluorescent seeding particles at micron scale. Through the synthesized particles, we are able to perceive and measure the flow patterns of fast dynamics. Illuminated by a normal continuous wave laser (CW) with a power of only 67 mW, we have created a fluorescent imaging speed up to 10,000 frames per second, which to the best of our knowledge, is the fastest record with a normal CW laser. We show that the introduction of CsPbBr3 QDs and the novel self-assembling method are keys to the final ultra-fast quantitative micro flow measurement, which reveals an alternative guide-line for the development of µPIV technology and shows a novel direction for fluorescent tracer particles as well.

The thesis is composed of 6 Chapters. The general fluid physics, experimental techniques utilized in this thesis are presented in Chapter 1 and Chapter 2 respectively. Chapter 3 explore
the emission mechanism of carbon dots. The demonstration in Chapter 4 imply the capability of carbon dots based microfluidic visualization, especially for the bio-microfluidics. To address the high speed quantitatively velocity field measurement issue in microfluidics, we document the CsPbBr3 Perovskite microparticles, as displayed in Chapter 5. The summary of the thesis and the prospects of microfluidics are shown in last Chapter (Chapter 6).
List of publications


3. Y. Huang#, **L. Xiao#**, T. An, W. Lim, T. Wong* and H. Sun*, ‘Fast dynamic visualizations in microfluidics enabled by fluorescent carbon nanodots’ Small, 2017, 13, 1700869. (# these authors contribute equally)


Citations to published work

1. Majority of Chapter 3 is adjusted from my publication:


2. Majority of Chapter 4 appears in my publication:

Chapter 1. Introduction

The integrated microelectronics have revolutionized our daily life, similarly, researchers especially in the fields of biology\(^1\), chemistry\(^3\) and pharmacy\(^4\) are interested in a question: whether it is possible to achieve the reaction system integration in a small chip, namely, lab on a chip, while with low cost and less reagent consuming\(^5\)? The ambition to achieving the “lab on a chip” motivate the development of the microfluidics\(^6\). Microfluidics, a platform or technology identified by the precise manipulation of fluid at micro scale, typically of 100 \(\mu m\), which aim to miniaturize both pumping and manipulation of the fluids, that is the reaction system, and provide an approach to accomplish the reaction system integration for biology, chemistry and pharmacy. Similarly to the microelectronics, the decreased size of microfluidics from the macro-scale to micro-scale allowing the possibility to achieve the integration\(^5\). In addition to the system integration requirement, microfluidics also originate from the demand of the bio-assays, which seek for a platform to operate the fluid at the cellular length scale, i.e. micro scale, and diagnostic analysis which look for efficient tool to accomplish the analysis but only consume a little sample volume\(^7\). In fact, we have already used some microfluidic products in a large scale such as liquid crystal displays, ink jet printers etc\(^8\).

Compared to traditional approaches, microfluidics manifest a variety of distinct advantages\(^5\),\(^6\),\(^9\)-\(^12\): 1. Similarly to the microelectronics, microfluidic platform owns the ability to operate various chemical and bio experiments automatically, rapidly and in parallel. 2. Considering the length scale is about 100 \(\mu m\), so the volume scale is the order of \(nl\), thus the sample volume required in microfluidics decreased considerably. 3. Because of the \(nl\) volume sample requirement, the cost of the reagents could be reduced substantially, and the signal collected from the costly sample can be enhanced greatly. 4. Differ from the macro-scale, fluid behaviors at micro scale are more predictable and controllable (the details will be discussed and presented in the following text) in both spatial and temporal dynamics, consequently the different micro
environments can be generated by the microfluidics, all of these can benefit for the cell-biology and pharmaceutics. 5. The reproducible and controllable of the fluid flows at micro-scale endow the microfluidic applications such as chemical species separation, detection and analyzing with high sensitivity and resolution. 6. The time consuming of reactions in micro-channels also reduced by virtue of the transport distance for the chemical species is decreased to micro scale.

Different from the microelectronics where the performance of most important components such as MOSFET are similar even the length scale reach to nanometer, the fluid behavior changes dramatically as the scale decrease from the macro-scale to micro-scale and the theoretical analysis of the scale dependent fluid physics will be given in next sub-section. At macro-scale, the gravity and inertial force will dominate the fluid behavior, resulting in the so called unpredictable turbulent flow, which is the physical word we perceive and populate . While as the fluid system shrike to micro scale, the surface to volume ratio enormously increased by several orders of magnitude, as a result, the interfacial/surface effect become ever more important. The fluid behavior at microscope is dominated by the viscosity, surface tension and diffusion, while the effect of the inertial force and gravity can be ignored (microfluidic flows reside in the low Re number condition, see next subsection for the details), resulting in a counterintuitive world and myriad microfluidic applications.

Generally, microfluidics can be divided into 4 categories: pined interface describing the parallel laminar flows generated by two immiscible flows; moving interface which illustrating the parallel laminar flows formed by two miscible streams; droplet, generated by two immiscible flows with one phase dispersed in another phase; even if the secondary interface (also called convention free flows in micro channels) also have some specific applications, while it is out of the scope of my thesis. In my thesis, I only focus on the first three microfluidic conditions, which are also the most important cases for microfluidic applications.
In addition to the versatile and fascinating applications, microfluidics also an efficient tool to explore the scale dependent interface properties, which endow microfluidics find applications in other fields such as system control, thermal management, energy generation, display technology etc. Moreover, as proposed by Javier Atencia & David J. Beebe, microfluidics offer a new dimension to the task or opportunity for everywhere the fluid is needed and used.

1.1 Fluid theory

In this part, we start from the basic conversation laws and assumptions to develop the general governing equations of fluid. In principal, all the fluid phenomena can be explained by the governing equations. In my thesis, I only consider the Newtonian fluids (as the most commonly utilized work fluids such as water, air etc. are Newtonian fluids), thus the governing equations become mass conservation law and Navier–Stokes equations.

Fluid: a material which can deform continually when exerted by a non-uniform stress, thus the kinematics of the fluid flow are consists of the fluid motion and deformation. Even through fluid is composed of discrete molecules, while in my thesis, I mainly concern the continuum depiction of fluid, which means that the macroscopic parameters such as velocity, pressure, temperature, concentration (all of them are the function of time and space) etc. have been utilized to dictate the kinematics of the fluid flow. The molecular scale dynamics is out of the scope of my thesis.

The responds of fluid to forces are fulfilled by deforming the fluid, and the forces on per unit area of the fluid is defined as fluid stress, which are related to the rate of strain or strain rate. The strain rate of a certain point is a description of the local velocity gradients, in other words, it dictate the deformation rate of the fluid component by a flow, thus fluid is regarded as inherently viscous. Differ from the fluid, the solid stress is associated with strain, which is a
depiction of its static deformation from its relaxed state, as a result, solids are always been considered elastic.

Form the preceding description, it's clear that the fluid behavior is closely related to the velocity gradient. For a general 3-dimensional (3D) flow, the velocity gradient is represented by the velocity gradient tensor, and which consists of two parts: strain rate tensor and rotation rate tensor.

We consider the general 3D flow case, in the Cartesian coordinates (unless otherwise specified, Cartesian coordinates are utilized in the thesis):

\[ \tilde{\mathbf{u}} = \mathbf{u}(u_x, u_y, u_z) \]
\[ u_x = u_x(x, y, z, t) \quad u_y = u_y(x, y, z, t) \quad u_z = u_z(x, y, z, t) \]

Thus, the strain rate tensor $\mathbf{\varepsilon}$ is defined as follows:

\[
\mathbf{\varepsilon} = \begin{pmatrix}
\varepsilon_{xx} & \varepsilon_{xy} & \varepsilon_{xz} \\
\varepsilon_{yx} & \varepsilon_{yy} & \varepsilon_{yz} \\
\varepsilon_{zx} & \varepsilon_{zy} & \varepsilon_{zz}
\end{pmatrix} = \begin{pmatrix}
\frac{\partial u_x}{\partial x} & \frac{1}{2} \left( \frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y} \right) & \frac{1}{2} \left( \frac{\partial u_z}{\partial x} + \frac{\partial u_x}{\partial z} \right) \\
\frac{1}{2} \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right) & \frac{\partial u_y}{\partial y} & \frac{1}{2} \left( \frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z} \right) \\
\frac{1}{2} \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right) & \frac{1}{2} \left( \frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z} \right) & \frac{\partial u_z}{\partial z}
\end{pmatrix}
\]

According to the definition, it's obvious that the strain rate tensor dictates the fluid deformation, which is determined by the local velocity gradients.

In addition to the strain rate tensor, the local velocity gradients also encompass other important information:
\[ \nabla \mathbf{u} = \begin{pmatrix} \frac{\partial u_x}{\partial x} & \frac{\partial u_x}{\partial y} & \frac{\partial u_x}{\partial z} \\ \frac{\partial u_y}{\partial x} & \frac{\partial u_y}{\partial y} & \frac{\partial u_y}{\partial z} \\ \frac{\partial u_z}{\partial x} & \frac{\partial u_z}{\partial y} & \frac{\partial u_z}{\partial z} \end{pmatrix} \]

\[ \nabla \mathbf{u}^T = \begin{pmatrix} \frac{\partial u_x}{\partial x} & \frac{\partial u_y}{\partial x} & \frac{\partial u_z}{\partial x} \\ \frac{\partial u_x}{\partial y} & \frac{\partial u_y}{\partial y} & \frac{\partial u_z}{\partial y} \\ \frac{\partial u_x}{\partial z} & \frac{\partial u_y}{\partial z} & \frac{\partial u_z}{\partial z} \end{pmatrix} \]

Where \( \nabla \mathbf{u}^T \) is the transpose of \( \nabla \mathbf{u} \). Thus \( \nabla \mathbf{u}^T \) can be rewritten as:

\[ \nabla \tilde{\mathbf{u}} = \frac{1}{2} \nabla \mathbf{u} + \frac{1}{2} \nabla \mathbf{u}^T + \frac{1}{2} \nabla \mathbf{u} - \frac{1}{2} \nabla \mathbf{u}^T \]

The symmetric part of \( \nabla \tilde{\mathbf{u}} \) is defined as \( \frac{1}{2} \nabla \mathbf{u} + \frac{1}{2} \nabla \mathbf{u}^T \), which is the formula of strain rate tensor \( \tilde{\varepsilon} \), and the antisymmetric part is defined as \( \frac{1}{2} \nabla \mathbf{u} - \frac{1}{2} \nabla \mathbf{u}^T \), we termed it as the rotation rate tensor \( \tilde{\omega} \). The strain rate tensor \( \tilde{\varepsilon} \) and rotation rate tensor \( \tilde{\omega} \) specify different types of fluid flows, as presented in the following subsection.

**Figure 1.1** Three kinds of fluid flows. (a) extensional flow, (b) shear flow, (c) rotation flow.
To understand the physical meaning of the strain rate tensor and rotation rate tensor, we consider the simple 2D flow, where $\varepsilon$ and $\omega$ are defined as:

$$\varepsilon = \begin{pmatrix} \frac{\partial u_x}{\partial x} & \frac{1}{2} \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right) \\ \frac{1}{2} \left( \frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y} \right) & \frac{\partial u_y}{\partial y} \end{pmatrix}$$

$$\omega = \begin{pmatrix} 0 & \frac{1}{2} \left( \frac{\partial u_x}{\partial y} - \frac{\partial u_y}{\partial x} \right) \\ -\frac{1}{2} \left( \frac{\partial u_y}{\partial y} - \frac{\partial u_x}{\partial x} \right) & 0 \end{pmatrix}$$

The diagonal component $(\frac{\partial u_x}{\partial x}, \frac{\partial u_y}{\partial y})$ of $\varepsilon$ dictate the fluid stretches or squeezes along the coordinate axes e.g. positive value for stretches, negative value for squeezing, as seen in figure 1.1 (a). In the condition of $u_x = x$, $u_y = -y$, the strain rate tensor $\varepsilon$ and rotation rate tensor $\omega$ are:

$$\varepsilon = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \quad \omega = \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix}$$

We call this kind of flow as pure extensional flow, as displayed in figure 1.1 (a), under the pure extensional strain, the rectangle shape reserved.

The off-diagonal component $\frac{1}{2} \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right)$ of $\varepsilon$ dictate the fluid skews termed as shear strain rate as seen in figure 1.1 (b). In the condition of $u_x = y$, $u_y = x$, the strain rate tensor $\varepsilon$ and rotation rate tensor $\omega$ are:

$$\varepsilon = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \quad \omega = \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix}$$

We call this kind of flow as pure shear flow, as displayed in figure 1.1 (b), under the pure shear strain, the rectangle skews to parallelogram.
To describe the rotation rate tensor, we define the vorticity as: \( \mathbf{\omega} = \nabla \times \mathbf{u} \), the vorticity has three elements which dictate the rotation magnitude and directions. If the vorticity has the formula \( \mathbf{\omega} = (\omega_x, \omega_y, \omega_z) \), the relationship between the vorticity and rotation rate tensor is:

\[
\mathbf{\omega} = \begin{pmatrix}
0 & -\frac{1}{2} \omega_z & \frac{1}{2} \omega_y \\
\frac{1}{2} \omega_z & 0 & -\frac{1}{2} \omega_x \\
-\frac{1}{2} \omega_y & \frac{1}{2} \omega_x & 0
\end{pmatrix}
\]

In the condition of 2D flow: \( u_x = -y, u_y = x \), the strain rate tensor \( \mathbf{\varepsilon} \) is zero while the rotation rate tensor \( \mathbf{\omega} \) is nonzero:

\[
\mathbf{\varepsilon} = \begin{pmatrix}
0 & 0 \\
0 & 0
\end{pmatrix}
\]

\[
\mathbf{\omega} = \begin{pmatrix}
0 & -1 \\
1 & 0
\end{pmatrix}
\]

As can be seen in figure 1.1 (c)\(^{19}\), the shape of the fluid flow is the same, while the flow direction rotated.

### 1.2 Conservation laws

The behavior of fluid is determined by the governing equations\(^{15, 19-21}\), thus in this subsection we will present the most important governing equations for fluid, namely the continuity equation (conversation law of mass) and Navier–Stokes equations (conversation law of momentum).

#### 1.2.1 Continuity equation

The continuity equation is established by the conservation of mass:

\[
\frac{\partial}{\partial t} \iiint_V \rho \, dv = -\iint_S (\rho \mathbf{u}) \cdot \mathbf{n} \, ds
\]

Where \( \rho \) density of the fluid, \( t \): time, \( \mathbf{n} \): outward normal vector of surface \( S \).
The left side of the equation is the mass change, the right side of the equation is the outward mass flux via the surface. Apply the divergence theorem, we obtain:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{u}) = 0 \quad 1.15$$

If the fluid is incompressible (which is appropriate for most applications cases), the density of the fluid is constant for both time and space, namely $\rho = \rho(\vec{r}, t) = C$, thus the mass conservation equation develops into:

$$\nabla \cdot \vec{u} = 0 \quad 1.16$$

1.2.2 Momentum conservation equations

We adopt two approaches to obtain the momentum conservation equations, where one is clear in mathematic derivation, while the other is clear in physics meaning.

We start from the Newton’s second law: $\vec{a} = \sum \vec{F}$, since $\vec{a} = \frac{d\vec{u}}{dt}$, so $m \frac{d\vec{u}}{dt} = \sum \vec{F}$. For fluid, the total forces are consist of body forces (gravity force, electromagnetic force etc.) and surface forces (pressure force and viscous force). And the surface forces are described by stress tensor $\sigma_{ij}$:

$$\begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{bmatrix} = \begin{bmatrix} \Omega_x \\ \Omega_y \\ \Omega_z \end{bmatrix} \quad 1.17$$

Where $\Omega_x, \Omega_y, \text{and } \Omega_z$ are the stress vectors on the planes perpendicular to the corresponding axes.

Thus, for a volume $dv = dx dy dz$ of fluid, the Newton’s second law on the x direction is:

$$\rho dv \frac{du}{dt} = \sum \vec{F}_{x, \text{body force}} + \sum \vec{F}_{x, \text{surface force}} \quad 1.18$$

$$= \vec{f}_{x, \text{body force}} dv + \sum \vec{F}_{x, \text{surface force}} \quad 1.19$$
Where $\bar{f}_{x,\text{body force}}$ is the body force density along $x$ axis.

To obtain the surface force on the $x$ direction, we utilize the following configuration:

![Figure 1.2 surface forces along x direction](image)

Combined with Taylor’s expansion formula, we obtain:

$$
\bar{F}_x = (\sigma_{xx} + \frac{dx\ \partial\sigma_{xx}}{\partial x}) dy dz \quad 1.20 \quad \bar{F}_x' = -(\sigma_{xx} - \frac{dx\ \partial\sigma_{xx}}{\partial x}) dy dz \quad 1.21
$$

$$
\bar{F}_y = (\sigma_{yy} + \frac{dy\ \partial\sigma_{yy}}{\partial x}) dx dz \quad 1.22 \quad \bar{F}_y' = -(\sigma_{yy} - \frac{dy\ \partial\sigma_{yy}}{\partial x}) dx dz \quad 1.23
$$

$$
\bar{F}_z = (\sigma_{zz} + \frac{dz\ \partial\sigma_{zz}}{\partial x}) dx dy \quad 1.24 \quad \bar{F}_z' = -(\sigma_{zz} - \frac{dz\ \partial\sigma_{zz}}{\partial x}) dx dy \quad 1.25
$$

So, the net surface force is:

$$
\sum \bar{F}_{x,\text{surface force}} = \bar{F}_x + \bar{F}_x' + \bar{F}_y + \bar{F}_y' + \bar{F}_z + \bar{F}_z' \\
= (\frac{\partial\sigma_{xx}}{\partial x} + \frac{\partial\sigma_{yy}}{\partial y} + \frac{\partial\sigma_{zz}}{\partial z}) dx dy dz \quad 1.26
$$

$$
= (\frac{\partial\sigma_{xx}}{\partial x} + \frac{\partial\sigma_{yy}}{\partial y} + \frac{\partial\sigma_{zz}}{\partial z}) dv \quad 1.27
$$

Thus,

$$
\rho \frac{du_x}{dt} = \bar{f}_{x,\text{body force}} + \frac{\partial\sigma_{xx}}{\partial x} + \frac{\partial\sigma_{yy}}{\partial y} + \frac{\partial\sigma_{zz}}{\partial z} \quad 1.29
$$

$$
\rho (\frac{\partial u_x}{\partial t} + u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y} + u_z \frac{\partial u_x}{\partial z}) = \bar{f}_{x,\text{body force}} + \frac{\partial\sigma_{xx}}{\partial x} + \frac{\partial\sigma_{yy}}{\partial y} + \frac{\partial\sigma_{zz}}{\partial z} \quad 1.30
$$
Similarly, we obtain

\[ x \text{ direction } \quad \rho \left( \frac{\partial u_x}{\partial t} + u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y} + u_z \frac{\partial u_x}{\partial z} \right) = \int f_{x, \text{body force}} + \frac{\partial \sigma_{xx}}{\partial x} + \frac{\partial \sigma_{xy}}{\partial y} + \frac{\partial \sigma_{xz}}{\partial z} 1.31\]

\[ y \text{ direction } \quad \rho \left( \frac{\partial u_y}{\partial t} + u_x \frac{\partial u_y}{\partial x} + u_y \frac{\partial u_y}{\partial y} + u_z \frac{\partial u_y}{\partial z} \right) = \int f_{y, \text{body force}} + \frac{\partial \sigma_{yy}}{\partial x} + \frac{\partial \sigma_{yx}}{\partial y} + \frac{\partial \sigma_{zy}}{\partial z} 1.32\]

\[ z \text{ direction } \quad \rho \left( \frac{\partial u_z}{\partial t} + u_x \frac{\partial u_z}{\partial x} + u_y \frac{\partial u_z}{\partial y} + u_z \frac{\partial u_z}{\partial z} \right) = \int f_{z, \text{body force}} + \frac{\partial \sigma_{zz}}{\partial x} + \frac{\partial \sigma_{xz}}{\partial y} + \frac{\partial \sigma_{yz}}{\partial z} 1.33\]

Vector form \[ \rho \frac{\partial \vec{u}}{\partial t} + \rho \vec{u} \cdot \nabla \vec{u} = \nabla \cdot \vec{\sigma} + \sum_i f_i 1.34 \]

We call the above expressions as Cauchy’s momentum equations.

The conservation momentum equation can also be obtained directly by analysis the momentum flux:

\[ \frac{\partial}{\partial t} \iiint_V \rho \vec{u} dv = \int \int \int_s (\rho \vec{u} \vec{u}) \cdot n ds + \int \int \int (\nabla \cdot \vec{\sigma}) ds + \sum_i \int \int \int f_i dv 1.35 \]

where \( \vec{u} \vec{u} \) is a second rank dyadic tensor.

The left-hand side of the equation is the momentum flux, the first term of the right-hand side is the momentum which flow out the selected volume by convection, the second term of right side is the momentum change by fluid stress, the third term of the right side is the momentum change by external body force.

Using the divergence theory, combing with the equation \((\alpha \beta) \cdot \gamma = (\beta \cdot \gamma) \alpha\), we obtain:

\[ \rho \frac{\partial \vec{u}}{\partial t} + \rho \vec{u} \cdot \nabla \vec{u} = \nabla \cdot \vec{\sigma} + \sum_i f_i 1.36 \]

Which is the Cauchy’s momentum equations. The momentum conservation equation dictates that the momentum flux (dynamic change) is the result of convection flow, fluid surface stress and external body force. In addition, the fluid stress comprise two parts: one is pressure stress (force) \( \vec{\sigma}_p \), where the direction of the pressure force is normal to the surface, and the magnitude
is independent on the velocity \( \vec{u} \), the other is the viscous stress (force) \( \vec{\sigma}_v \), where both the normal and tangent directions have non-zero component, and the magnitudes are related to the velocity \( \vec{u} \) distribution:

\[
\vec{\sigma} = \vec{\sigma}_p + \vec{\sigma}_v
\]

And the pressure stress can be dictated by the following form:

\[
\vec{\sigma}_p = -p \vec{\delta} = \begin{pmatrix} -p & 0 & 0 \\ 0 & -p & 0 \\ 0 & 0 & -p \end{pmatrix}
\]

where \( \vec{\delta} \) is intensity tensor.

While, until now, we still need the information of \( \vec{\sigma}_v \), which is related to the strain rate tensor. However, the relationship between the strain rate tensor and viscous stress cannot be derived from any conservation laws, instead they are connected by some constitutive relations. And the most important one is the Newtonian relation.

If the strain rate tensor is linearly related to the viscous stress, we call these fluids as Newtonian fluids:

\[
\vec{\sigma}_v = 2 \mu \vec{\varepsilon} = \begin{pmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{pmatrix} = \begin{pmatrix} 0 & \mu \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right) & \mu \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right) \\ \mu \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right) & 0 & 2 \mu \frac{\partial u_y}{\partial y} \\ \mu \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right) & \mu \left( \frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial y} \right) & 0 \end{pmatrix}
\]

Where \( \mu \) is viscosity [Pa s] of the fluid, which is independent to the velocity.

The total surface stress tensor can be described as:

\[
\vec{\sigma} = 2 \mu \vec{\varepsilon} - p \vec{\delta}
\]
So

\[ \nabla \cdot \sigma = \nabla \cdot (\sigma + \sigma_r) = 2 \mu \varepsilon - \rho \ddot{\delta} = -\nabla P + \nabla \cdot \mu \nabla \dot{u} \quad 1.41 \]

The Cauchy’s momentum equation become the Navier–Stokes equation:

\[ \rho \frac{\partial \ddot{u}}{\partial t} + \rho \ddot{u} \cdot \nabla \ddot{u} = -\nabla P + \nabla \cdot \mu \nabla \ddot{u} + \sum f_i \quad 1.42 \]

Navier–Stokes equation is the governing equation for the Newtonian fluid, which have drawn much of scientists’ and engineers’ attention as the most important fluids water and air are Newtonian fluid. In my thesis, I only focus on the Newtonian fluid, which means that all the behaviors of fluids utilized in my experiments can be described by the Navier–Stokes equations.

1.3 Boundary condition

To solve the governing equations, the boundary conditions are also required. For fluids, the boundary condition can be divided into two parts: velocity boundary condition and stress boundary condition.

We consider the boundary which mass cannot cross (this assumption is correct if there is no chemical reaction and physical phase change), then the normal velocity relation can be obtained from the conservation of mass:

For a control volume across fluid 1 and fluid 2 (figure 1.3), the conservation law of mass is described as:

\[ \ddot{u}_1 \cdot n - \ddot{u}_2 \cdot n = 0 \quad 1.43 \]
Then the boundary condition for the normal velocity is given by:

\[ u_{1n} = u_{2n} \]  \hspace{1cm} 1.44

The tangential boundary condition cannot be derived from any conservation laws, thus the hypothesis have been specified for specific fluid problems, in the condition of our dynamic fluid problems, generally we assume that the tangential velocity are also continues:

\[ u_{1t} = u_{2t} \]  \hspace{1cm} 1.45

This assumption is consistent with the experimental observations in our fluid dynamic cases, while it must be emphasized that one cannot expand this assumption to universal cases.

Before we start to discuss the stress boundary condition, we first define the surface tension:

For a system which comprises two phases 1 and 2, the surface tension of the interface is given by:

\[ \gamma_{12} = \left( \frac{\partial G}{\partial S} \right)_{T,P} \]  \hspace{1cm} 1.46
Where $G$ is the Gibbs free energy of the whole system, $S$ is the surface of the interface.

According to the definition, it's clear that the surface tension is a dictation of the additional energy due to the existence of the surface, thus surface tension can also be regarded as the force per unit length, which tends to minimize the interface surface.

The surface tension is closely related to the stress boundary condition: The force balance on surface is achieved by the fluid stress and the interface stress, namely surface tension. The fluid stress for the Newtonian fluid is given by:

$$\sigma \cdot n = -p n + \mu \frac{\partial \vec{u}}{\partial n} + \mu \nabla u_n$$  \hspace{1cm} 1.47

And the surface tension engenders 2 parts of interfacial stress: one is tangential stress: $$\gamma \nabla \cdot n$$ originate from the surface tension gradient, the other is the normal stress, $$-(\nabla \gamma - (\nabla \gamma \cdot \vec{n}))n$$ come from the curvature of the interface.

Thus, the stress boundary condition is given by:

$$\sigma_2 \cdot n - \sigma_1 \cdot n = (\gamma \nabla \cdot \vec{n})n - (\nabla \gamma - (\nabla \gamma \cdot \vec{n}))n$$  \hspace{1cm} 1.48

Decompose the boundary condition to normal and tangential direction, we obtain:

$$\sigma \cdot n = (\gamma \nabla \cdot \vec{n})n$$  \hspace{1cm} 1.49
$$\vec{t} = -(\nabla \gamma - (\nabla \gamma \cdot \vec{n}))n$$  \hspace{1cm} 1.50

For Newtonian fluid flow, we can rewrite the vector-tensor form as the scale form:

$$P_1 - P_2 + 2\mu_2 \frac{\partial u_{n2} \cdot \vec{n}}{\partial n} - 2\mu_2 \frac{\partial u_{n1} \cdot \vec{n}}{\partial n} = \gamma \left( \frac{1}{r_1} + \frac{1}{r_2} \right)$$  \hspace{1cm} 1.51
$$\mu_1 \frac{\partial u_{n1}}{\partial n} + \mu_1 \frac{\partial u_{n3}}{\partial t} - \mu_2 \frac{\partial u_{n2} \cdot \vec{n}}{\partial n} - \mu_2 \frac{\partial u_{n2}}{\partial t} = -(\nabla \gamma - (\nabla \gamma \cdot \vec{n}))$$  \hspace{1cm} 1.52

Where $r_1, r_2$ are the radii of the curvature of the interface of phase 1 and phase 2 respectively.
1.4 Microfluidics: low Reynolds number flow

For the incompressible Newtonian fluid flow, the behaviors of the fluids are dictated by the governing equations, that is, conservation of mass and conservation of momentum equations:

\[ \nabla \cdot \vec{u} = 0 \]  \hspace{1cm} (1.16)

\[ \rho \frac{\partial \vec{u}}{\partial t} + \rho \vec{u} \cdot \nabla \vec{u} = -\nabla P + \mu \nabla^2 \vec{u} + \sum_i f_i \]  \hspace{1cm} (1.42)

Unfortunately, until now, there is still no general solutions for the Navier–Stokes equations. While the condition become different when the length scale of the fluid is reduced to micro-scale (typically 100 µm), namely microfluidics. In order to understand microfluidics, we start from nondimensionalizing the Navier–Stokes equations.

The nondimensional variables are defined and represented by primed properties:

\[ x' = \frac{x}{L}, \quad y' = \frac{y}{L}, \quad z' = \frac{z}{L} \]  \hspace{1cm} (1.53)

\[ \text{thus} \quad \nabla' = \frac{\nabla}{1/L}, \quad \nabla^2 = \frac{\nabla^2}{1/L^2} \]  \hspace{1cm} (1.54)

We also normalize the velocity and time scale:

\[ \vec{u}' = \frac{\vec{u}}{U}, \quad t' = \frac{t}{t_c}, \quad P' = \frac{P}{P_c} \]  \hspace{1cm} (1.55)

Where L, U and P_c are the characteristic length, velocity and pressure of the system respectively. t_c is the characteristic time. If the boundary are fixed, \( t_c = \frac{L}{U} \); while if the boundary are change rapidly, and \( t_{bc} \) is the minimal time interval of two boundary sates, then \( t_c = \frac{L}{U} \) for \( \frac{L}{U} < t_{bc} \); \( t_c = t_{bc} \) for \( \frac{L}{U} > t_{bc} \), in other words, the characteristic time is the fastest time scale of the system.

With these definitions, we substitute and rewrite the Navier–Stokes equations into a nondimensional from (we ignore the body force here):
Define Reynolds number as \( \Re = \frac{\rho UL}{\mu} \), thus the nondimensional Navier–Stokes equations can be rewritten as:

\[
\Re \frac{L}{Ut_c} \frac{\partial \vec{u}}{\partial t} + \Re \vec{u} \cdot \nabla \vec{u} = -\frac{P}{\mu U} \nabla P + \nabla^2 \vec{u} \quad 1.57
\]

The Re number is one of the most important parameters in fluidics\(^5\),\(^14\),\(^19\), which categorize different fluid regimes. For a specific geometry, if the Re number is small, we call the flow as laminar flow i.e. once the boundary are given, a single (deterministic) flow could be observed experimentally. The laminar flow field is stable with the condition that the boundary condition is steady, that is to say, the laminar flow is mainly determined by the boundary condition which manifest stability to perturbations\(^19\). And thus it’s predictable and controllable for a given boundary condition (later we will explain more about it). While when the Re number is large enough, the laminar flow change to turbulent flow, the behavior of the fluid is mainly controlled by the motion of the fluid, namely the inertial force. The turbulent flows are random and unsteady even through the boundary conditions are steady. As a result, we cannot predict the fluid flow from the boundary conditions, usually the turbulent flow is unpredictable. If the Re number is in the range between the laminar flow and turbulent flow, we call these flows transitional flows, which have some steady mathematical solutions of the Navier–Stokes equations, nonetheless these steady flows cannot be observed experimentally, as the flows are still unsteady. Generally\(^5\),\(^19\), we regard the flow as laminar flow if \( \Re < 2300 \), totally turbulent flow for \( \Re > 4000 \), and transitional flow for \( 2300 < \Re < 4000 \).

We rewrite the Reynolds number as:

\[
\frac{\rho UL}{\mu} \frac{L}{Ut_c} \frac{\partial \vec{u}}{\partial t} + \frac{\rho UL}{\mu} \vec{u} \cdot \nabla \vec{u} = -\frac{P}{\mu U} \nabla P + \nabla^2 \vec{u} \quad 1.56
\]
Thus, the Re number is also an estimation of the relative importance of the inertial forces and viscous forces. Let’s consider the microfluidic flow: typically for water as the fluid, \( \rho \sim 10^3 \text{kg/m}^3 \), characteristic velocity \( U \sim 100 \text{\mu m/s} \), characteristic length \( L \sim 100 \text{\mu m} \), viscosity \( \mu \sim 10^{-2} \text{g/cm} \cdot \text{s} \), thus the Re number is the order of \( \sim 10^{-2} \). For microfluidics, the low Re number guarantees the flow in micro channel is located at the laminar flow regimes, where the inertial effect can be ignored and the viscous will dominate the fluid behavior, as a consequence, the microfluidic flow become predictable and controllable.

If the Re number \( Re \ll 1 \) (this assumption is valid for microfluidics), and there is no extra external body forces, then, the only body force is gravity. However, in the condition of low Re, compared to the viscous force, the gravity (inertial force) can be ignored, namely, the body force term is zero. We consider the nondimensionalized Navier–Stokes equations:

\[
\text{Re} \cdot \frac{L}{Ut_c} \frac{\partial \tilde{u}}{\partial t} + \text{Re} \tilde{u} \cdot \nabla \tilde{u} = -\frac{P_L}{\mu U} \nabla P + \nabla^2 \tilde{u}
\]

which becomes:

\[
\text{Re} \cdot \frac{L}{Ut_c} \frac{\partial \tilde{u}}{\partial t} = -\frac{P_L}{\mu U} \nabla P + \nabla^2 \tilde{u}
\]

Return to the dimensional form:

\[
\rho \frac{\partial \tilde{u}}{\partial t} = -\nabla P + \mu \nabla^2 \tilde{u}
\]
Steady flow

Define Strouhal number $St$ as $St = \frac{Ut}{L}$. Thus, for the steady flow, $St=1$, the nondimensional Navier–Stokes equations become:

\[
\text{Re} \cdot \frac{\partial \vec{u}}{\partial t} + \text{Re} \vec{u} \cdot \nabla \vec{u} = -\frac{PL}{\mu U} \nabla P + \nabla^2 \vec{u} \quad 1.63
\]

Combined with the low Re number assumption ($Re \ll 1$), both the unsteady term ($\frac{\partial \vec{u}}{\partial t}$) and nonlinear/convection ($\vec{u} \cdot \nabla \vec{u}$) term can be ignored, thus the Navier–Stokes equations become Stokes equations:

\[
0 = -\frac{PL}{\mu U} \nabla P + \nabla^2 \vec{u} \quad 1.64
\]

Rewrite the Stokes equations to dimensional form, it becomes:

\[
\nabla P = \mu \nabla^2 \vec{u} \quad 1.65
\]

1.4.1 Immiscible fluids parallel laminar flows: vertical pinned interfaces

At macro scale, the fluid behavior is dominated by the inertial force, e.g. gravitational force. So the final state of two immiscible fluids such as water and mineral oil mixture is determined by the gravity, namely the relative density of the fluids. Mineral oil will locate at the upper part due to its lower density and water will reside in the lower part because of its larger density. The water and mineral oil are separated by a horizontal interface, as is observed in our daily life. While when the scale decreases to micrometer, things are different. As we discussed above, the fluid behavior at micro-scale is determined by the viscous forces and surface tension, as a consequence, when two immiscible fluids contact with each other, they will generate a vertical interface instead of a horizontal interface, as illustrated in figure 1.4, which is unavailable at macro scale. Combining with the laminar flow essence of microfluidics, endowing the opportunity to precisely control the vertical interface which can act as a virtual wall.
The vertical pinned interface at micro scale has promoted a variety of applications\textsuperscript{22-28}. The surface patterned micro channel has been utilized to generate the water/air interface\textsuperscript{22}, which can be used to degas under normal pressure, as illustrated in figure 1.5 (a), the large surface to volume ratio at micro scale ensure the high degas (gas exchange) efficiency, in fact, it’s a mimic of our lungs’ alveoli. In addition to degassing, this microfluidic system can also be used to adjust the gas component in liquid by virtue of the high gas exchange efficiency.

By properly designing and pretreating the micro channels, the parallel laminar flows can be employed to the finish multi-phases reaction. Juta Kobayashi et al\textsuperscript{23} have realized an efficient hydrogenation reaction using the microfluidic devices, as illustrated in figure 1.5 (b). The large surface area at micro scale guarantee the sufficient interaction between the gas (hydrogen), liquid (substrate) and solid catalyst, and the shrinked size of the micro channel decrease the required diffusion length of the hydrogen molecular, all of these facilitate the reaction.
When all of the immiscible phases are consist of liquid such as water and organic solvents, it can produce a vertical organic/water membrane, which can be used to the interfacial investigation. Mariana Surmeian et al.\textsuperscript{24} have explored the molecular transport dynamics when passing an organic membrane (figure 1.5 (c)). In addition to the molecular transport dynamics, Tatsuo Maruyama et al.\textsuperscript{25, 26} also explored the metal ions transport/separation by using the water/n-heptane/water microfluidic system. Moreover, the immiscible parallel laminar flow interface can also be used to demonstrate the chemical and bio reactions. Hideaki Hisamoto and co-workers\textsuperscript{27} have synthesized membrane structures from the organic/aqueous/organic parallel laminar flow system by means of interfacial polycondensation reaction, as depicted in Figure 1.5 Various applications based on immiscible parallel laminar flows\textsuperscript{22-24, 27}
figure 1.5 (d). Tatsuo Maruyama et al\textsuperscript{28} have demonstrated the enzymatic degradation process at the interface between two phases by utilizing the microchip.

1.4.2 Mixing problems at micro-scale and Pe number

Different from the macro scale (high Re number), the mixing dynamics of fluids at micro scale (low Re number) shows different behaviors, causing the unique microfluidic mixing problems. There are two passive approaches which contribute to the fluids flux into or out of a volume, i.e. diffusion and convection. Diffusion is the net fluid flux due to the existence of the local chemical concentration gradient. The direction of the net flux is opposite to the gradient, namely, the chemical species flux from the high concentration to low concentration. Even if the diffusion is the result of molecular scale thermal fluctuation, the ensemble effect can be dictated by the macroscopic constitutive relation, that is, Fick’s law:

\[ \vec{j}_d = -D\nabla c \]

(1.66)

Where \( \vec{j}_d \) is the flux density of diffusion, D is the diffusivity of the chemical species in the solution, and \( \nabla c \) is the concentration gradients of the chemical species. In addition to the thermal fluctuation triggered passive diffusion transport, the redistribution of the chemical species can also be accomplished via convection, and the convection flux density \( \vec{j}_c \) is determined by the velocity and species concentration:

\[ \vec{j}_c = uc \]

(1.67)

We assume that 1. The concentration of the chemical species are relative low, thus the fluid properties such as density, viscosity, surface tension etc. are not affected by the scalar. 2. The active transport approach such as external electric field is not involved in this subsection. 3. The source term is ignored i.e. no chemical reaction happens. Thus, we can derive the passive scalar conservation equation (in my thesis, the scalar refers to species concentration):
The scalar conservation equation describes that for a given volume $V$, the chemical species change in the volume is the result of the convection flux and diffusion flux out of the volume (see figure 1.6). Combined with the divergence theory, we can obtain:

$$\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c = D \nabla^2 c$$

1.70

Figure 1.6 Species fluxes for a Cartesian control volume.

Similar to the Navier–Stokes equations, we nondimensionalize the passive scalar conservation equation:

$$\frac{L}{U_t} \frac{\partial \hat{c}}{\partial t} + \hat{\mathbf{u}} \cdot \nabla \hat{c} = \frac{1}{UL/D} \nabla^2 \hat{c}$$

1.71

Define $\text{Pe} = \frac{UL}{D}$ as the mass transfer Peclet number, and $t_c = \frac{t}{U}$ for steady flow; $t_c = t_{bc}$ for rapidly changed boundary condition, similar to the Navier–Stokes equations.

For steady flow, the nondimensionalized passive scalar conservation equation become:
Thus, it’s become clear that the Pe number is an evaluation of the relative importance (magnitude) of the passive diffusion and convection for a given system. For a low Pe number system, the mass/species transport can accomplish efficiently via passive diffusion \( \left( \frac{1}{Pe} \nabla^2 \vec{c} \right) \), while for a high Pe number system, the mass transport is mainly carried out by convection \( (\vec{u} \cdot \nabla \vec{c}) \).

Before we apply the passive scalar equation into microfluidics and discuss microfluidic mixing problem, we first briefly discuss physics of mixing dynamics. The passive mixing dynamics is determined by the passive scalar convection-diffusion equation, and we can gain insight into the mixing process if we split the convection and diffusion effects into two individual steps even though it’s not the actual mixing process\(^5\).\(^{19}\). The passive diffusion transport the chemical species via scale gradients, the transport or mixing efficiency is determined by the diffusion length scale defined as \( L_d = \sqrt{D \tau} \). The diffusion length scale describes the distance where the chemical species have diffused from the start point to the position where the concentration is nearly half of the total equilibrium concentration at time \( t \). On the contrary, for a given distance \( L \), the time required to complete the mixing by passive diffusion is proportional to \( \frac{L_d^2}{D} \). However, the existence of passive convection can hugely increase the mixing efficiency by shorting the diffusion length scale \( L_d \). Figure 1.7 is an example of shorting the diffusion length scale by convection. The red ribbon region contains the chemical species, it will mixing with the green band, the convection triggered the stretch (extensional strain) and folding(rotation), thus dramatically decrease the effective diffusion length scale as can be seen in figure 1.7. Thus, passive diffusion only need to accomplish the much shorter length scale to achieve the full mixing. That’s why we always stir or shake the different liquids to mix them in our daily life.
The process of shorted diffusion length always take place in high Re number flow. For the high Re number flow, the nonlinear /convection term in the Navier–Stokes equations cannot be ignored and play an important role in mixing dynamics, consequently the convection will decrease the diffusion length and accelerate the mixing process. While in the condition of low Re number microfluidics, the nonlinear /convection term can be ignored, there is no efficient convection flows, thus in addition to some specific designs, the microfluidic mixing is very slow. The conclusion can be also obtained from the Pe number argument. For microfluidics, the typical channel width \( W \) is \( \sim 100 \mu m \), the characteristic velocity \( U \) is \( \sim 100 \mu m/s \), and the diffusivity \( D \) is \( \sim 10^{-10} - 10^{-16} m^2/s \), thus the Pe number is in the range of \( 10^2 - 10^9 \), which absolutely reside in the high Pe number range. Considering the definition of the Pe number, the high Pe number means that the mass transport or mixing is mainly dominated by the convection process, while the low Re number specified that the nonlinear/convection \((\vec{u} \cdot \nabla \vec{u})\) effect in the microfluidic flow is inconsequential, therefore the passive mixing in microfluidics is incapable of achieving the full and rapid mixing.
Figure 1.7 Stretching and folding process can decrease the effective diffusion length.

In the condition of low Re number and high Pe number microfluidics, the convection is insufficient, thus the time required to mix for a distance L (in microfluidics, the mixing distance is always the channel width W) is the scale of $t_{mi} = \frac{L^2}{D} = Pe \frac{L}{U}$, during the time $t_{mi}$, the distance which the fluid in the micro channel has flowed is $l_c = Ut_{mi} = PeL$, and $l_c$ is the minimal length requirement of the channel for the full mixing take place. Table 1\textsuperscript{5, 19} are the minimal times and channel lengths required for some chemical species to fulfil mixing in water (channel width (W): 100 $\mu m$, which is the required diffusion length scale $L_d$ (see figure 1.8), velocity: 100 $\mu m/s$).

![Figure 1.8 Micro channel for the mixing to happen](image)

Table 1 The minimal time and channel length required for some chemical species to fulfil mixing in water

<table>
<thead>
<tr>
<th>Chemical species</th>
<th>Diffusivity(m$^2$/s)</th>
<th>Time required to full mixing (s)</th>
<th>Distance of the fluid flow(m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>$1\times10^{-9}$</td>
<td>$10^1$</td>
<td>$10^{-3}$</td>
</tr>
</tbody>
</table>
The above discussion indicate that the mixing process occurs at micro scale mainly via diffusion due to the high Pe number. Depending on different application scopes, this property can be beneficial or not. Microfluidic systems are usually utilized to perform chemical species separation, diffusion dynamic investigation, extraction component from mixture etc., the laminar flow with suppressed mixing can promote these applications for the reason that faster mixing will result in the separation much more difficult. While on the contrary, in the condition of chemical reactions or bio reactions/assays, such as material synthesis, DNA microarrays etc., we mainly focus on the reaction dynamics, thus the total mixing is required before the reaction happen, namely the mixing time scale must be shorter than the reaction time scale. The slow mixing of the laminar flow thus hinders these applications, hence both passive (e.g. Taylor dispersion\textsuperscript{29}, Rotary mixer\textsuperscript{30} and Chaotic advection\textsuperscript{31}) and active (e.g. applying an external electric field\textsuperscript{32}) methods have been proposed by researchers to facilitate the mixing process, as will be discussed in the flowing main text.

The miscible parallel laminar flow also provokes tremendous applications. One typical example is the so-called T-sensor as seen in figure 1.9 (a), which consists of two streams. The two side streams contact at the T junction and flow into a main channel, forming a predictable diffusive interface owing to the laminar essence. The basic work principle of the T sensor is as follows: one side flow comprises the analyte components/species, the other side flow incorporates the fluorescent linked analytical reagents where the emitted fluorescent light act
as the signal readout. By making using of the T sensor, both concentration analysis, diffusion and chemical reaction dynamics monitor, and molecular mass sensing have been demonstrated by different research groups. Furthermore, Anson Hatch et al. even realized the competitive diffusion immunoassay (DIA) by means of micro T sensor, the validation of the T sensor based DIA was confirmed by the successfully analysis of phenytoin (a useful small drug molecular) in the blood sample.

A T and a reversed T junction can form an H junction, as illustrated in figure 1.9 (b). The H junction can be used to perform the filtration/extraction while without membranes, thus it is also called H filter. Two streams contact each other in the upper T junction and flow into the main channel. One side flow (see the right side flow in figure 1.9 (b)) is the sample stream which contains a variety of sizes of chemical species, the other side flow (the left side flow in figure 1.9 (b)) is the particle free extraction flow. According to the Stokes–Einstein equation, the diffusivity of the materials manifest inverted correlation to particle size, consequently the Pe number and main channel length (which is linearly proportional to the Pe number) required to diffuse from the sample stream to particle free extraction flow are various for different particles. In other words, by properly controlling the main channel length and the velocity, small Pe number particles will diffuse to the extraction stream before the separating of the sample flow and extraction flow, while the large Pe number particles cannot diffuse to the extraction stream, which still reside in the sample flow. At the end of the main channel, another T junction is employed to separate the sample flow and extraction flow, where the extraction flow mainly encompasses small particles, which is collected and continuously flow for the next analysis, while the sample flow still consists of all kinds of particle species.

Compared to membrane based filter, the H filter can avoid contaminates and destruction of the samples, and the only work principle is the different mobility along the channel width. All of these triggered the H filter based bio-selection and separation. Brenda S. Cho et al. have
used the H filter to separate sperm, as illustrated in figure 1.9 (c). Compared to the nonmotile sperm (generally the sperm is dead, at least the quality of the sperm is low) which can only move via passive diffusion, the motile sperm can move to the extraction phase quickly as in addition to passive diffusion, it can also move via actively swimming. Similarly, the extracted motile sperm can be separated from the nonmotile sperm and collected for the further purpose.

It’s obvious that the microfluidic H filter conserve the sperm vitality during the extraction, thus guaranteeing the following applications, which is unavailable for the membrane based filtration. Moreover, Eric A. Schilling et al. also operated the protein extraction from the bacterial cells (E. coli) by means of the microfluidic H filter.

**Figure 1.9** Applications of miscible parallel laminar flows. (a) T sensor, (b) H filter, (c) Motile sperm extraction.

The above-mentioned applications are operated at the intermediate Pe number conditions, while the high Pe number parallel laminar flow also finds various applications. The non-mixing streams can be employed to construct the micro electrode within the channels, which can contribute to the electric field manipulation of the fluids. In addition, the pattered micro channels have also been realized by means of high Pe number flows. Different regions of the
high Pe number parallel flows can form steady different micro environment\textsuperscript{45}, thus cells which reside in these micro environments shall display different behaviors. Consequently, locally probing the cell response to different micro environments become available in the micro channels, which have already been demonstrated by several research groups\textsuperscript{46-48}.

### 1.4.3 Droplet and Ca number

The continuous laminar flow allows the precise control of the fluid at micro-scale, while the ‘lab on a chip’ applications require complex and parallel experiments integrated into a single chip. Consequently, the size of the chip must linearly increase as the complexity of the system increases, which will limit the scope of the microfluidic applications. To address this issue, the droplet based microfluidics have been proposed\textsuperscript{49-52}. The micro droplet can be performed as micro reactors, thus different reactions can be carried out separately and parallelly inside different micro droplets reactors. So droplet based microfluidics are capable of executing numerous experiments simultaneously but without increasing the system complexity and size, which can contribute to achieve the ‘lab on a chip’. In addition to the system integration, the generation of micro droplet are also required by pharmaceutical and food industries, e.g. both drug\textsuperscript{53}, DNA\textsuperscript{54}, cell\textsuperscript{55} etc. related bio-capsulation, emulsions, homogeneous micro particle generation\textsuperscript{52} and etc. have been demonstrated successfully by means of droplet based microfluidic approach. All of these triggered researchers to investigate the droplet based microfluidics, as the microfluidic system can generate droplets with highly controllable and reproducible.

Differ from the continuous laminar flow, droplet based on microfluidics concentrate on the generation and applications of the discrete volumes of fluid i.e. micro droplet generated by employing immiscible fluids (discrete phase and continuous phase). Compared to continuous
flow, the discrete character of the droplet enables to manipulate it individually, as a result, the droplet based micro-reactors can be independently transported, reacted, and analyzed.

The droplet based microfluidics are still in the condition of low Re number, which means compared to viscous force, the inertial force can be ignored. Aside from the viscous force, the surface tension also become crucial (the value can be measured by pendant drop method) as the size decrease to micro scale. And both interfacial tension and shear viscous force can regulate the micro droplets behaviors with a competing manner. So, the behavior of the micro droplet is determined by the relative capability of these two forces, defined as Capillary number:

\[
Ca = \frac{\mu U}{\gamma}
\]

where \(\mu\) is the viscosity of the continuous phase, \(U\) is the characteristic velocity of the continuous phase, \(\gamma\) is the surface tension between the continuous phase and discrete phase. A low value of Ca number implies that the shear viscous force is relatively small compared to surface tension, while large Ca number result in the shear viscous dominated flow. The droplet size displays inverted relation to the Ca number as the higher interfacial shear viscous force will reduce the size of the micro droplet. We want to emphasize that for a certain system, the droplet can form only when the system Ca number great than a critical Ca Number \(Ca_c\) (the absolute values vary for diverse system), otherwise it will form continuous laminar flow.

The pre-requisition of making use of the droplet based microfluidic platform is to generate the discrete micro droplet with controllable size, shape and monodispersity. After more than two decades of efforts, researchers have proposed a variety of approaches to generate droplet such as T-junction, flow-focusing, electrohydrodynamic (EHD), dielectrophoresis (DEP) etc. While in my thesis, I only focus on the T-junction and flow-focusing approaches, which are also the most common adapted methods by many researchers due to their advantages such as easy of chip fabrication, precise fluid control etc.
**T junctions**

T junction based micro droplet generation was first proposed by Thorsen et al.\textsuperscript{61} the configuration of the T junction is illustrated in figure 1.10 (a), the dispensed phase flow into the junction from the left inlet channel, while the continuous phase flow into the junction from the lower main channel. The dispersed flow and continuous flow intersect perpendicularly in the junction, resulting in an interface at the junction. The dispensed phase will flow into the main channels as it flow continuously, while once the viscous shear force exerted by the continuous phase stream overcome the surface tension between these two fluids, the droplet of the dispersed phase will generate. The break up process are briefly as follows: the viscous shear force exerted by the continuous phase resulting in an instantaneous pressure gradient, which lead to the head of the dispersed phase extend into the main channel, the extension stops upon the neck of the dispersed phase thinning, and finally break up into a micro droplet.

**Flow focusing**

The flow focusing geometry was first proposed by Anna et al.\textsuperscript{58} and Dreyfus et al.\textsuperscript{62} The geometry configuration is depicted in figure 1.10 (b). It can be seen that the inner dispersed phase is squeezed by another 2 outer continuous phase flows. Differ from the T junction configuration, flow focusing utilize symmetric shearing to generate droplet by means of using two continuous elongational flow streams. And the droplet is generated by the break-up of the thin filament of the inner disperse phase flow. Because of the existence of a third phase, compared to T junction, the flow focusing configuration manifest better controllability and stability of droplet generation.
In both cases, the immiscible disperse phases and continuous phases are driven into the micro channels independently and encounter at the junction part. And the size of the droplet could be controlled by the channel geometry (e.g. channel width), flow rate, and relative viscosity between the immiscible continuous phase and disperse phase. And the surfactants always been utilized in microfluidics, which can reduce the surface tension between the continuous phase and disperse phase, thus facilitate the droplet formation. Besides, the existence of the surfactant can prevent the coalescence of the generated droplets, thus stabilize the droplets/emulsions. By proper controlling the conditions, the highly mono-dispersed micro droplets with the size variations less than 1% have been reported by various research groups.

We must emphasize that the surface properties of the channel walls are crucial to generate micro droplet as the interfacial effect become dominate at the micro scale. To prevent the discrete droplet from adhering/wetting to the channel walls and promote the wetting between the continuous phase and the channel walls, usually the discrete phase and the channel have the opposite surface properties, while the continuous phase has the similar surface properties with the channel walls. For example, when we generate water droplet in oil (water acts as the disperse phase, oil acts as the continuous phase), the hydrophobic channel walls are...
designed e.g. PDMS, while when generate oil droplet in water, the hydrophilic channel walls are required, which can be achieved by surface coating.

Droplet based microfluidics have stimulated innumerable applications, such as chemical or material synthesis\textsuperscript{66-71}, microparticle synthesis\textsuperscript{3, 72, 73}, microchannel resonator\textsuperscript{2}, microdroplet-based PCR sequencing\textsuperscript{1}, single-cell Chromatin immunoprecipitation sequencing\textsuperscript{74}, protein crystallization\textsuperscript{75}, separate circulating tumour cells from cancer patients\textsuperscript{76}, Single-cell analysis and sorting\textsuperscript{77}, Drug Discovery and Medical Diagnostics\textsuperscript{78-80}, Drug Delivery\textsuperscript{53, 81, 82} and etc., as pointed out by Ansgar Huebner\textsuperscript{52}, ‘there is an application sea for micro-droplets’, and there also exists numerous review papers\textsuperscript{50, 51} which discuss the droplet based properties and applications. In addition, more and more fascinating and even revolutionary droplet based applications are continuously appearing.

1.5 Significance and motivations of this study: Explore new fluorescent nano materials to visualize microfluidics

As we discussed previously, both the parallel laminar flows and droplet microfluidics own countless fascinating applications, while if we carefully check these applications, we will notice that most of the applications are based on the proper fluorescent materials as the fluorescent signal can increase the signal to noise ratio and/or the fluorescent signal itself is regarded as the readout for most of applications such as bio-analysis, velocity measurement, mixing dynamics exploration etc. Generally, microfluidics utilize fluorescent materials by means of three approaches: Interface capture, velocity measurement and signal readout. While the currently available fluorescent materials or fluorescent seeding particles cannot meet the requirement of the microfluidic applications.
1.5.1 Bio-microfluidic visualization: Carbon dots

For bio applications, especially for the living cell related applications, the typically used rhodamine series dyes cannot be employed by virtue of their toxicity, and the bio-compatible fluorescent materials such as proteins, the cost is not acceptable especially when considering mass product. To address these issues, we first employed the carbon dots as the fluorescent marker to trace the fluid interface dynamics.

Carbon dots have become the centre of numerous scientific investigations since its discovery\textsuperscript{83, 84} due to the conspicuous merits such as superior bio-compatibility for both in vitro and in vivo, chemical/colloidal stability, photo stability, easy of surface function group modification, these along with the low cost and environmental friendly allows carbon dots to find numerous applications in fields of biology, optoelectronics, chiral phonics, catalyst etc.\textsuperscript{84-86} It’s obvious that the further exploration and practical applications of carbon dots built upon the appearance of the stable, fast and above all low cost synthesis approaches. And fortunately researchers have gained sufficient progress for carbon dots synthesis during past 15 years. Until now, numerous carbon dots synthesis approaches comprising various fascinating fast and one-step avenues have been published by different researchers. Thus, carbon dots have become the center of substantial research endeavours to exploit the alternate to take the place of the currently utilized unsatisfied fluorescent materials.

On the whole, there are two routes to synthesize carbon dots: top-down methods and bottle-up approaches, and each of them encompass many sub-categories. Top down route consists of Arc-discharge approach\textsuperscript{83}, Laser ablation/irradiation method\textsuperscript{84}, Electrochemical method\textsuperscript{87}, Chemical exfoliation avenue\textsuperscript{88} etc., while bottle up route comprise Thermal Pyrolysis method\textsuperscript{89}, Hydrothermal approach\textsuperscript{90}, Microwave heating approach\textsuperscript{91}, Anchor/Support based method\textsuperscript{92}, metal–organic frameworks (MOFs) template based method\textsuperscript{93} etc. While in all synthesis approaches, the intriguing microwave facilitated carbon dots fabrication method have drawn
much of attentions by virtue of its striking merits such as less time consumption, fast and homogeneous heating, low cost etc.. Thus this novel fabrication method opens a new avenue to synthesize carbon dots\textsuperscript{94}, such as, Sourov \textit{et al} \textsuperscript{95} and Qu \textit{et al} \textsuperscript{96} have achieved the relative high PL quantum yield green emission carbon dots by means of micro wave heating method, blue light emission carbon dots with PL quantum yield more than 40\% (excitation wavelength: 360 nm ) is published by Cui’s group\textsuperscript{97}. Furthermore, even deep ultraviolet light and near infrared emission from carbon dots also realized by Hao’s group\textsuperscript{98} and Lin’s group \textsuperscript{99} by adopting microwave approach. Thus, in our experiments, we also employ the microwave assisted method to synthesize carbon dots.

Both the further making better use of carbon dots and adjusting the photo-physical properties requires the deep understanding of carbon dots PL mechanism. However, the exact PL mechanism of carbon dots are still controversial even thorough some progress have been achieved during the past decade. It is well accepted that carbon dots synthesized from different methods present some similar properties such as several nano-meter size, relative strong PL emission, abundant surface function groups, excellent bio-compatibility etc., however, the entirely opposite/conflicting features have also been observed from different kinds of carbon dots, both the photo stable\textsuperscript{94, 100} and photo blinking\textsuperscript{101-103}, pH inertness\textsuperscript{104} and pH dependent PL\textsuperscript{105, 106}, quantum size dependent\textsuperscript{104, 107-109} and size independent PL\textsuperscript{88, 110}, excitation wavelength dependent\textsuperscript{110} and independent PL\textsuperscript{104, 111} \textit{etc}. have been published by different researchers. All of these imply that carbon dots represent a much complex system than expected. Both band gap emission with quantum confinement effect\textsuperscript{104}, Surface states emission\textsuperscript{112}, Molecular fluorophores and carbogenic core emission\textsuperscript{113}, Polycyclic Aromatic Hydrocarbon molecular emission\textsuperscript{100}, Slowed solvent relaxation or solvatochromic shift\textsuperscript{114}, Surface dipole emission centre\textsuperscript{115}, Aggregate emission centre\textsuperscript{116} etc. have been proposed to
reveal the carbon dots emission, while none of the proposed mechanisms can consistently interpret all optical phenomena observed from C-dots.

To utilize carbon dots to visualize the microfluidic interface, it’s necessary and pre-requisite to give insight into the carbon dots emission, thus, we employed the polarization spectroscopy, electric-field modulation spectroscopy, power dependent measurement and time resolved PL measurement to disclose the carbon dots emission, our results offer clear and strong evidence that carbon dots emission originate from the self-trapped exciton, where both the momentum and energy relaxation of the photo generated hot carriers (electrons and holes) are greatly suppressed. Based on our self-trapped exciton model, all the optical properties observed from C-dots including the steady-state and time-resolved spectral data can be interpreted convincingly.

After gaining the knowledge of carbon dots emission, we have also validated the merits of carbon dots, such as chemical inertness, photo-stability, and excellent bio-compatibility. The following microfluidic demonstration indicated that carbon dots display good performance in microfluidic flow visualization, both the mixing dynamics induced by electrohydrodynamic instability, cladding phase control, droplet/complex droplet generation dynamics have been captured and analyzed by using carbon dots as the fluorescent tracer, as presented in Chapter 4. In addition, for the first time we synthesized and introduced C-dots micro sized seeding particles (CDsP) for quantitative measurements of flow fields at microscale, where the carbon dots based seeding particles also characters relative high imaging intensity, excellent biocompatibility and a substantially lower price compared to the conventional fluorescent tracers. Our results demonstrate that fluorescent carbon dots can serve as an advantageous whereas affordable material for visualization in microfluidics which may not only find plenty of applications in biology, chemical synthesis and medicine industry but also enable quantitative studies of fluid dynamics at microscale.
1.5.2 High speed flow field measurement: CsPbBr3 quantum dots and self-assembled CsPbBr3 micro seeding particles

The high speed quantitative measurement of the flow field holds significant impact to both the fundamental mechanism studies and the practical applications in the field of microfluidics. Micro particle image velocimetry (µPIV) which is considered as the most powerful and widely accepted quantitative measurement technology has been playing a critical role in both fundamental investigations and practical applications such as the well-known micro droplets, encapsulations, high efficiency sorting, and cell sequencing, all of which character themselves in fast dynamics. To match fast dynamics in microfluidics, high-speed µPIV with fluorescent seeding particles is highly demanded. Unfortunately, current fluorescent tracer particles suffer from low emission intensity as most of them are synthesized by coating fluorescent materials (dyes or semiconductor quantum dots) onto the surface of non-fluorescent spheres, which limits the imaging speed and shows vulnerability in capturing fast dynamics. Thus, current high speed µPIV technologies try to elevate the speed dominantly by adopting more powerful illumination systems, specifically, lasers. This approach inevitably creates issues such as the high cost and undesirable heating effect, albeit it merely achieves feasible measurement speed in the order of a few thousand frames per second (FPS) which is still far from the required measurement speed.

Since year 2015, all-inorganic cesium lead halide perovskite CsPbX3 (X=Cl, Br, and I) have been attracting increasing attentions because of their unique properties such as the high photoluminescence quantum yield (PLQY), relatively improved thermal and moisture stabilities, tunable emission wavelength, long carrier time and narrow emission peak. They are showing great potentials in solution-processable optoelectric devices, for instances, solar cells, light emitting diode (LED) and lasers. The merits of inorganic perovskite QDs render themselves the potential in high speed flow field measurement at micro-scale. However, there are two important issues that hinder the
applications of halide perovskite QDs in flow measurement at microscale. First, perovskite QDs cannot be directly used in flow field measurement because the diameter of halide perovskite QDs usually lies in several to tens of nanometers while the sizes of particle used in μPIV should be in the order of micron or submicron to avoid random blinking, Brownian motion and weak emission signal from a single nanoparticle. Second, the inorganic perovskite QDs are highly unstable once encountered an aqueous phase. In order to address these two issues, we implemented a new approach of self-assembling the cesium lead halide perovskite CsPbBr₃ QDs into micron scale particles in mineral oil. The synthesized particles are protected by the oil phase and thus can be applied in aqueous based phases. Different from the commercially available fluorescent particles synthesized by a layer of coating on the surface, our self-assembled particles are completely composed of the perovskite QDs, thus they are brighter and can achieve a much higher imaging speed. Moreover, the cost of our approach is much lower than the commonly used fluorescent seeding particle method, as the coating process is not required.
Chapter 2. Experimental Techniques

2.1 Sample synthesis

2.1.1 Carbon dots synthesis

The microwave assisted method have been employed to synthesize carbon dots for the reason that microwave can provide homogeneous, rapid heating, while low cost. Briefly, for carbon dots sample A synthesis, 0.5 g glucose was dissolved into 3 ml DI water, followed by 5 min microwave heating, the transparent solution changed to faint yellow solid which indicated the formation of carbon dots. The solid carbon dots were dissolved into 15 ml water to form the carbon dots solution, and the purification of carbon dots solution is accomplished by 0.45 μm filter with 3 replicate purification process, the final faint yellow transparent solution is collected for further use (figure 2.1 (a)). For carbon dots sample B, the procedures are similar, while the reaction reagents include both glucose and urea where the glucose act as the carbon source and the urea offer the nitrogen source to achieve the doping. Typically, 0.5 g glucose and 0.5 g urea mixture was dissolved into 3 ml DI water forming transparent solution. After 5 min microwave heating, the transparent solution transformed into dark yellow, and finally dark brown solid, meaning the formation of carbon dots. The solid sample was dissolved into 150 ml DI water, followed by purification by means of 0.45 μm filter with 3 replicates (figure 2.1 (b)). For optical spectroscopy measurement, both the carbon dots samples are drop-casted on transparent sapphire substrates. All the materials glucose (formula: C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) and urea (formula: NH\textsubscript{2}CONH\textsubscript{2}) are from sigma Aldrich.

2.2.2 C-dots and C-dots micro particles synthesized for microfluidic visualization

Carbon dots sample B was utilized as the fluorescent material to capture the interface of microfluidics. The synthesis procedures are similar as presented above. The only difference is
that in this experiment we use 30 ml DI water to dissolve the synthesized carbon dots sample by reason of the higher carbon dots concentration, as seen in figure 2.1 (c).

Still the carbon dots sample B is utilized to synthesize carbon dots based micro particle. Typically, the synthesized carbon dots solid sample was dissolved into 45 ml DI water, followed by purification with 0.45 μm filter for 3 replicas. 15 ml of the synthesized carbon dots solution was collected and mixed with 0.3 ml DI water which contain 10 wt% surfactant (SDS, from Sigma Aldrich), then 0.1 g of porous polystyrene particles (form EPRUI nanoparticles & Microspheres Co. Ltd) was added into the mixture, the existence of the surfactant can prevent the particle aggregation. After 72 hours of continuous stir mixing at room temperature, the carbon dots based seeding/micro particle was generated. The solid particle can be obtained by centrifuging the mixture (7500r, 15min) for 2 times. The particle concentration must meet the requirement of flow field measurements which can be controlled by properly dissolving the solid particles into certain amount of DI water. In our experiment, the carbon dots seeding particles were dissolved into 2 ml aqueous solution as depicted in figure 2.1 (d).

Figure 2.1 Imagines of carbon dots samples. (a) carbon dots sample A. (b) carbon dots sample B dissolved in 150 ml DI water. (c) carbon dots sample B dissolved in 45 ml DI water. (d) carbon dots sample B based seeding
particles. (a),(b) are the carbon dots samples for the optical spectroscopy characterization, and the (c),(d) are the carbon dots samples for the microfluidic applications.

2.2.3 Synthesis of the CsPbBr$_3$ perovskite QDs

Our solution based CsPbBr$_3$ perovskite QDs were prepared by reacting the Cs-oleate with a PbBr$_2$ in a solvent of octadecene$^{124,126}$. It consists of three steps as follows:

**Cs-oleate preparation:**

Cs$_2$CO$_3$ (0.814g, Sigma-Aldrich), octadecene (ODE) (40ml, Sigma-Aldrich) and Oleic acid (OA) (2.5ml, Sigma-Aldrich) were loaded into a 100 ml three neck flask. The mixture was dried/degassed about 1 hour at 120°C, then we increase the temperature to 150°C using N$_2$ as the protection gas. After a certain time, all Cs$_2$CO$_3$ will react with OA. The preheating (150 °C) is required before injection.

**Synthesis of CsPbBr$_3$ QDs:**

ODE(15 ml), Oleylamine (OAm)(3ml, Sigma-Aldrich), OA(1.5 ml), and PbBr$_2$ (0.2g) were loaded into a 100 ml three neck flask and degassed at 120°C for 20 min. After the PbBr$_2$ was totally dissolved into the solution, the mixture was heated to 170°C under N$_2$, followed by quick injection of 0.66ml Cs-oleate solution (0.125 M in ODE). The reaction mixture was quickly cooled by ice-water bath after 10 s of the injection.

**Purification of CsPbBr$_3$ QDs:**

8 ml of acetone was added into 4 ml of the crude solution to precipitate the CsPbBr$_3$ QDs. The CsPbBr$_3$ QDs are collected after centrifugation at 8500 rpm for 5 min. Finally, hexane was utilized to dissolve the precipitate (10 mg/ml).
2.3 Steady state PL measurement

The set-up of the steady state PL measurement is illustrated in figure 2.2. The continuous wave (CW) He-Cd laser, which can emit two kinds of light i.e. 442 nm and 325 nm, have been utilized to excite the sample. The chopper-lock-in amplifier technique can increase the signal to noise ratio dramatically, endowing the system with high sensitivity and resolution, thus can detect very weak PL signal. The PL signal are collected by two collimated lenses and finally guided into the monochromator whose basic work principle is the diffraction grating. The split monochromatic light is detected and analyzed by the PMT (Photomultiplier tube) or CCD, resulting in the PL spectra.

![Figure 2.2 Set-up for steady states PL measurement.](image)

For polarized PL measurement, half-wave plate is adapted to rotate the polarization of the excitation light, and polarizer is placed on the PL collecting optical path to select the different directions of polarization light. In all cases, another half wave-plate is needed to adjust the direction of polarized PL light to eliminate the polarization effect of the measurement system, as depicted in figure 2.3.
2.4 Time resolved PL

Time resolved PL technique is a useful tool to explore the temporal PL spectra dynamics which is unavailable for steady state PL spectroscopy. Typically, a short pulse is utilized to excite the sample, the generated electron hole pair recombination resulting in the photoluminescence. The measurement of the PL spectra evolution with time can offer the information of transition energies (PL spectra) and the fluorescent lifetime. And the streak camera system is employed to measure the PL spectra dynamics in the range of ns or ps for the reason that the traditional spectrum analyser such as gated intensified CCD cannot provide such high time resolution.
The basic work principle of the streak camera is presented in figure 2.4. The short pulse generated luminescent light pass the spectrograph which convert the PL to monochromatic light. Then the following photocathode transform the monochromatic light into the corresponding electrons, where the number of the converted electrons proportional to the light intensity. When the converted electron streams pass a pair of sweep electrodes, a time dependent voltage, which is synchronized with the laser source, is exerted to sweep electrodes, generating high speed electron sweep.

![Figure 2.4 Time-resolved PL measurement by streak camera](image)

The electrons released by the streak unit travel through a time dependent field which is synchronized with the laser excitation. Consequently, the electrons which dictate the early component of the incident light pulse describing shorter lifetime of the PL signal located at the upper part, while the electrons which characterize the later component of the incident light pulse depicting longer lifetime of the PL signal located at the lower part, thus the time evolution is converted into spatial distribution. After the signal amplification by means of micro channel plate (MCP) and bombarded against the phosphor screen, the final signal is outputted. The relative brightness of the output images describes the corresponding fluorescent light intensity,
the horizontal axis dictate different wavelengths of emitted light and the vertical axis of the images illustrate the time evolution of the PL.

2.5 Home-made microfluidics fluorescent imaging system

![Diagram of the home-made microfluidic fluorescent imaging system]

Figure 2.5 Home-made microfluidic fluorescent imaging system.

The CW He-Cd laser is adapted as the light source, after passing the microchip, an objective lens is placed to enlarge and image the microfluidic system. Still, two collected lenses are needed to guide the fluorescent light into the high-speed camera. Due to the existence of the long pass filter, only the fluorescent light can enter the camera. As a result, the fluids which contain fluorescent material are bright while other regions are dark, and the intensity contrast contribute to the interface capture, velocity measurement etc. We must emphasize that the collimated light is crucial for the high-quality imaging. The whole system is depicted as figure 2.5.
2.6 Microchip Fabrication methods

We employ the lithographic method for engraving the design on to the silicon wafer, where we adopt a negative photoresist Su8 from Microchem. The Su 8 is spin coated on a silicon wafer. The thickness of the Su 8 is controlled by the spin coating protocol (basically the coating speed). Then the wafer with Su 8 on top is exposed into a UV light environment for the polymerization process. A photo mask is used to achieve a controlled polymerization (figure 2.6 (a)). After going through the developing process, only the portion shined by the UV light is left on the silicon wafer (figure 2.6 (b)). Followed by the polydimethylsiloxiane (PDMS, Dow Corning Sylgard 184) filling and curing process, the design of the structures is successfully copied, as depicted in figure 2.6 (c). Next, we demold the PDMS layer from the silicon wafer and conduct the oxygen plasma bonding with one slice of microscopy glass substrate spin coated with one thin layer of PDMS to ensure uniform surface properties. The microchip is finalized by placing into an oven for 2 hours heating at temperature of 120 °C to stabilize the surface property.

For facilitating the formation of complex droplet (double emulsion), microchip with two distinct yet controlled surface properties (hydrophobic and hydrophilic properties) is necessary. As the PDMS layer shows a uniform hydrophobic surface property, we modify the surface from the hydrophobic to hydrophilic by using the UV initialized polymerization as plotted in figure 2.6 (e).

Figure 2.6 Microchip fabrication process.
Chapter 3. Carbon dots emission mechanism: self-trapped exciton

3.1 Introduction

Carbon dots (C-dots) as a new kind of carbon based fluorescent material have drawn extensive attention and triggered numerous investigations since their first discovery over a decade years ago. Compared to conventional semiconductor quantum dots or fluorescent dyes, C-dots exhibit excellent biocompatibility, reflected not only by C-dots materials themselves, but also by their synthesis process. Furthermore, the successfully explored one-step synthesis method resulted in extremely low cost for C-dots fabrication, which together with the photo-stability, chemical inertness, and surface function group flexibility, endows C-dots the enormous application potential in bio-imaging, drug-delivery, photo-catalyst, light emitting diodes (LEDs), flexible display etc. More importantly, the abundant and reproducible row materials for C-dots guarantee the huge likelihood for large-scale practical applications in industry.

To make better use of C-dots, in particular for the optoelectronic applications, fully understanding of the C-dots emission mechanism is essential. C-dots synthesized by different synthetic approaches, precursors, and post treatment manifested dissimilar optical performances, indicating that C-dots shall be a more intricate system than what expected. As a consequence, until now, the genuine origin of C-dots emission remains an open question. So far, surface states emission, intrinsic band emission, triple ground states emission, dipole emission involving electron-phonon coupling, transition from surface electrons to valance holes etc. have been proposed to explain the photo-physical properties of C-dots. A distinctive feature of C-dots emission is excitation dependent photoluminescence (PL), i.e. the emission spectra will shift as the excitation wavelength changes. Researchers attributed this unique phenomenon to different possible origins such as multi-emission centers, C-dots size
distribution, slow solvent relaxation and the existence of multi-aggregation. However, to the best of our knowledge, none of the proposed mechanisms can consistently interpret all optical phenomena observed from C-dots.

Here, we uncover the emission mechanism of C-dots by utilizing the polarization anisotropy spectroscopy, electric-field modulation spectroscopy and time resolved PL measurement, from which we provide clear evidence that C-dots emission results from self-trapped excitons. Based on the self-trapped exciton model, all the emission properties of C-dots including the steady-state and time-resolved spectral data can be interpreted consistently. Thus our investigation may engender new insight about the physical origins of C-dots luminescence.

3.2 Results and discussion

The C-dots samples were prepared by employing the reported microwave assisted methods with slight modification. The details of the synthesis procedure can be seen in the Chapter 2.1.1. Two kinds of samples were prepared and investigated comparatively. Sample A is synthesized from glucose which contains C, H, and O elements. Urea as extra N source which can modify the optical properties of C-dots by doping has been utilized to synthesize sample B. The XRD spectral data of the sample A and B are shown in figure 3.1 (a) and (b) respectively, which indicate that both carbon dots samples are amorphous as no effective XRD

![Figure 3.1 XRD spectra of carbon dots. (a), (b) for sample A and B respectively.](image)
signal was observed from the samples. Both of the carbon dots samples display no obvious Raman peaks associated with carbon, as can be seen in figure 3.2, which is consistent with the XRD spectra data where carbon dots own amorphous structure. The broad spectra in figure 3.2 come from the fluorescence of carbon dots.

![Raman spectra of carbon dots. (a), (b) for sample A and B respectively.](image)

Figure 3.2 Raman spectra of carbon dots. (a), (b) for sample A and B respectively.

The absorption spectra of C-dots are presented in figure 3.3 which showed a broad absorption range from UV to near IR. When excited by a 442 nm laser, both C-dots samples show bright emission, as shown in figure 3.3. Compared to band edge emission, the line-shape of C-dots emission spectra show quite different features: 1) The emission of C-dots starts from the excitation wavelength, and emission range is so broad, from near excitation wavelength (450nm) to 700nm, the total photon energy spans about 1 eV\textsuperscript{143}. 2) In the whole broad spectra, the intensity of every emission wavelength is of the same order of magnitude, which is different from the band edge emission where the emission intensity near the peak is dominant \textsuperscript{144}-\textsuperscript{146}.

When the samples are excited by monochromatic light, the non-thermal equilibrium hot carriers will be generated, the following relaxation by scattering and/or collision with carriers(electrons and/or holes) may lead to the thermal equilibrium distribution of hot carriers, resulting in the normal luminescence(i.e. luminescence which come from the thermalized electron-hole recombination)\textsuperscript{147}. Other than the normal luminescence from thermal equilibrium carriers, hot
carriers luminescence in which the photons are emitted before the thermal equilibrium is established may also exist. In the absence of localized mode in the excited states, the intensity of the hot luminescence is extremely weak (many orders of magnitude lower) relative to normal luminescence as the relaxation time is much shorter than the luminescence lifetime. It is plausible to speculate that the C-dots emission (excited by 442 nm) is a kind of hot carrier luminescence especially for the emission near 450 nm, since it seems impossible for the hot carriers to be totally thermalized after just undergoing 50 meV relaxation process.

![Figure 3.3](image)

**Figure 3.3** (a) Absorption and PL (excited by 442 nm) spectra of sample A. (b) Absorption and PL (excited by 442 nm) spectra of sample B.

Hot carrier luminescence is different from the normal luminescence, which takes place in the excited states during the relaxation process, producing a unique feature: the system is not fully degraded and the information (momentum, spin, energy, etc.) of the electronic system induced by the excitation photons are partially/totally reserved. On the contrary, the existence of the conserved information (for example momentum ($P$)) of the carriers will explicitly demonstrate the hot carrier properties.

Polarization spectroscopy offers the opportunity to investigate the momentum and energy relaxing dynamics. When sample is illuminated by linear polarized light, the momentum of the hot carriers will be aligned, resulting in the anisotropic momentum distribution. The
corresponding principles are governed by the transition density matrix combined with the angular momentum distribution restricted by the dipole approximation. The anisotropic distributed momentum will evolve the linear polarized emission as the selection rules are identical for the emission and absorption. Nevertheless, the subsequent decay process after the excitation will randomize the aligned momentum distribution of carrier ensemble by collision or emission phonons leading to an isotropic momentum distribution. Thus, the polarized emission can only be observed for the carriers which have not lost their momentum alignment. In other words, the carriers must be hot carriers which reserve the momentum anisotropy caused by the linear polarized light. The degree of polarization of the emission light will decrease quickly with the increase of the emission wavelength because lower energy carriers

Figure 3.4 Emission anisotropy and degree of linear polarization of film Sample A (figure (a), (c)) and film sample B (figure (b), (d)).
undergo further relaxation, and the polarization will totally disappear for the thermalized carriers as the momentum distribution become isotropic\textsuperscript{153}. It is clear that polarization spectroscopy can be used as a useful tool to probe the momentum and energy relaxation dynamics of the photo-induced hot carriers and give significant information about the electronic structure properties.

Polarization spectra are strongly related to the sample circumstance. There are remarkable differences between the solution phase and solid phase samples, as the Brownian rotation in

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure3.5.png}
\caption{(a), (c) Excitation anisotropy and degree of linear polarization for sample A, (b), (d) are the results for sample B. Both the excitation anisotropy and polarization show similar behaviors of emission. Excitation wavelength: 442 nm.}
\end{figure}

solution is inevitable\textsuperscript{154, 155}, which means that the anisotropy spectra in C-dots solution as conducted by Syamantak Khan, Mariia O. Dekaliuk and Arjun Sharma\textsuperscript{114, 140, 156} result from
both the decay process by carriers in the electronic structure and Brownian rotation. For the purpose of unveiling the intrinsic electronic structure and the momentum decay dynamics of C-dots, we firstly explored the anisotropy of C-dots films which perfectly eliminates the Brownian rotation, just as in other material systems like amorphous carbon, $\text{As}_2\text{S}_3$, black phosphorus, and graphene oxide, GaAs and $\text{etc}^{152, 157-160}$. The emission anisotropy of C-dots films as a function of photon energy are shown in figure 3.4 (a) and 3.4 (b) where the vertical axis represents the angle between the polarization of the excitation and emission light in which the electric field of the excitation is fixed. It can be clearly seen that both C-dots samples A and B show high emission anisotropy. In order to reconfirm the anisotropy of the C-dots emission, we also carry out the excitation anisotropy measurement (see figure 3.5 (a) and 3.5 (b)), in which the electric field of the emission which we collect is fixed, and the electric vectors of the excitation light are rotated by a half wave-plate. The excitation anisotropy of C-dots showed similar behaviors with the emission anisotropy, which explicitly manifests the anisotropic feature of C-dots emission.

We define the degree of linear polarization as $P = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$ for the sake of quantitative description of the emission anisotropy of C-dots, where $I_\parallel$ is the emission intensity whose polarization or electric vector parallel to the excitation light (0 degree), $I_\perp$ is the emission intensity whose electric field is perpendicular to the excitation light (90 degrees), the results are depicted in figure 3.4 (c) and 3.4 (d) (Similarly, The degree of the excitation linear polarization is defined as $P_{\text{ex}} = \frac{I_{\text{ex}\parallel} - I_{\text{ex}\perp}}{I_{\text{ex}\parallel} + I_{\text{ex}\perp}}$, where the $I_{\text{ex}\parallel}$ is the emission intensity when the polarization of the excitation light is parallel to the collection emission polarization (0 degree), $I_{\text{ex}\perp}$ is the emission intensity where the polarization of the excitation light is perpendicular to the emission polarization(90 degrees). Both the excitation anisotropy and the polarization (see figure 3.5 (a) - (d)) of C-dots are similar to the emission results, which reconfirm the anisotropy.
properties of C-dots. The degree of linear polarization is large and even reaches to 0.5 at the
instant of excitation for both C-dots samples, and continually decrease as the energy difference
between the excitation light and emission light increases, which is the result of momentum
relaxation of the hot carriers as the emission energy decrease. There still exist large
polarization values (about 0.4 for film sample A, 0.25 for film sample B) even though the energy
difference between the excitation and emission light is more than 1 eV, which undoubtedly
indicates that it is the hot carriers that contribute to the C-dots emission.

The polarization spectra are determined by the degree of the momentum anisotropy $\beta$ which is
related to the energy relaxation time $\tau_e$, and momentum anisotropy disappear time $\tau_{p2}$. The
general expression for the momentum anisotropy parameter $\beta$ evolution during the decay
process is as follows:

$$\beta = \beta_0 \exp\left(-\int_{\tau_{p2}}^{\tau_e} \frac{dE}{E}\right) \quad (3.1)$$

Collision of the carriers, scattering by the impurities, and phonon emission will lead to the
decrease of $\beta$, and usually $\beta$ decrease to zero rapidly as the momentum relaxation time of hot
carriers is much shorter (several order of magnitude) than the lifetime of emission. The
situation is quite different for C-dots, the momentum relaxation time of hot carriers is almost
similar to or even larger than the fluorescence lifetime as we can still observe the high linear
emission polarization in the red emission region, which implies that the high anisotropy
momentum distribution can survive in the decay process. Besides the uncommonly high linear
polarization, C-dots also show distinct PL intensity distribution compared to band emission.
The emission intensity of C-dots are comparable in the whole emission spectra, while in the
case of band emission the overwhelming majority of the luminescence is the normal
luminescence and distributed in the near band edge as the vibrational time is much shorter
(several orders of magnitude ) than the lifetime of the luminescence. The high emission
polarization and the unique emission line shape unambiguously demonstrate that C-dots emission is different from the band edge emission, more specifically, both the momentum relaxation, energy relaxation, vibrational relaxation are heavily suppressed. It’s reasonable to propose that there are some localized modes contributing to the C-dots emission which confine/impede the movement of the hot carriers, resulting in the subdued decay process.

**Figure 3.6** Emission anisotropy and degree of linear polarization of solution Sample A (figure (a), (c)) and solution sample B (figure (b), (d)). Excitation light: 442 nm.

We also carried out the C-dots solution emission anisotropy and polarization measurement as depicted in figure 3.6 (a)-(d), and the excitation anisotropy and polarization are presented in figure 3.7 (a)-(d) which also shows a similar performance to the emission results. Compared to the C-dots film, the polarization spectra of C-dots solution exhibit different properties as we predicted previously. The polarization value for C-dots solution is lower, and the spectra are
more complicated: the polarization value first decrease followed by gradually increase as the emission energy decrease. The variance between the film and solution samples can be counted for the result of Brownian rotation perturbation to the pure polarization spectra of C-dots, which is consistent with the published results\textsuperscript{114, 155}.

Figure 3.7 (a),(c) Excitation anisotropy and degree of linear polarization for sample A aqueous solution, (b),(d) are the results for sample B aqueous solution. Both the excitation anisotropy and polarization show similar behaviors of emission. Excitation wavelength: 442 nm

As was shown earlier, the emission of fluorescence material (such as GaAs quantum wells, Carbon nanotube, porous silicon, C\textsubscript{60} etc.) will be quenched when an high electric field was applied, which is related to the hot carriers properties\textsuperscript{161-164}. The electric field can produce the separation of the hot carriers by altering the polarization of the hot carriers distribution along
or opposite the electric field, which results in the modulation of the energy states or the non-radiative decay rate\textsuperscript{165, 166}. Besides, electric field may cause spatial shift of the hot carriers, which diminishes the overlap of the associated wave functions (thus decrease the radiative recombination rate)\textsuperscript{165, 167}. J. A. Kash and Hideki Koyama also attributed the field induced quenching to the wave function leakage and the quantum tunneling effect\textsuperscript{161, 168}. Here we attempt to explore electric-field modulation of photoluminescence to elucidate the emission mechanism of C-dots. The results of the electric-field modulation for C-dots are anomalous as shown in figure 3.8 (measurement configuration: The samples are subject to an electric field in a Voigt geometry, where the electric field is perpendicular to the excitation light). It is found

**Figure 3.8** Electric-field modulation of sample A (figure (a)) and sample B (figure (b)). Insert is the emission spectra of C-dots sample. Excitation light: 442 nm

that both of the C-dots samples do not show any electric quenching even if the electric field reaches 1500V/cm. Both the intensity and the line shape of the emission spectra are nearly identical with and without electric fields. The results imply the strong localization properties of the emission centers which provide a strong localized potential field so that the high electric field cannot “affect” the hot carriers and quench the emission of C-dots by the above modulation mechanisms, such kind of phenomenon has been similarly observed in the other localized emission materials\textsuperscript{157, 162, 169, 170}. The lack of electric field quenching is consistent
with our assumption that the existence of localized mode which contributes to the C-dots emission. Besides, the photo-stable properties in high electric field also endow C-dots with the potential applications, for example, tracing the microfluidic mixing dynamics which requires the stable emission under high electric fields.\textsuperscript{171-173}

After exhausted discussion, we can assert the localized mode essence for C-dots emission. However different kinds of localized model will lead to disparate optical performances. Defect/impurities is a common localized state which exists in many materials.\textsuperscript{174-177} For defect emission, there are two types of defect energy states transitions: one is localized defect to band (free carriers) transition (or band to defect transition), the other is the defect to defect transition. Considering the extremely high linear polarization value (up to 0.5 for both sample A and B), defect-band transition seems impossible to explain the high polarization values as the free carriers in the band will loss the momentum anisotropy very fast (the time scale is ps, which is much shorter than the PL lifetime ns), so the only possible configuration is defect-defect transition, in which both the photo-excited electrons and holes are confined. However, for defect to defect transition, it must have complex configuration to match the optical properties of carbon dots, such as broaden emission (over 1 eV), excitation dependent PL etc. More importantly, it will be saturated easily as the density of defect is relative low. Another important localized model is self-trapped exciton which has been suggested to contribute to the photoluminescence in SiO$_2$, C$_{60}$, PbBr$_2$, alkali halide crystals etc.\textsuperscript{178-181}

Alexander S. Urban et al. attribute pyrene which have the self-trapped exciton property to the short wavelength range excitation of C-dots.\textsuperscript{100} However, there was no clear evidence to prove the existence of self-trapped excitons in C-dots in their study. Self-trapped excitons and defects present different response to the increased excitation power, where the defect emission tends to saturate due to the relative low density of states. In contrast, self-trapped excitons are expected to demonstrate linear response to the increased excitation power as the number of the
produced excitons are proportional to the excitation power\textsuperscript{179, 180}. Here we performed power dependent PL measurements and the results are presented in figure 3.9 (excitation wavelength 442 nm, CW laser).

![Figure 3.9](image)

**Figure 3.9** Power dependent PL of sample A (figure (a), (c)) and sample B (figure (b), (d)). The unit of excitation power in figure (a) and (b) is mW/cm\(^2\). Excitation light: 442 nm

The absence of saturation behavior even upon the excitation power density as high as 1800 mW/cm\(^2\) for both C-dots samples does not agree with defect emission, confirming that the emission from C-dots originates from self-trapped exciton recombination. For the first time, our results provide clear and convinced evidence of the existence of self-trapped excitons in C-dots.
Figure 3.10 (a) Time-resolved spectroscopy measurements of film Sample A. (b) Time-resolved spectroscopy measurements of film sample B.

As compared to itinerant hot free carriers, photo-generated localized exciton excited states in C-dots result in the reduced relaxation and degradation process which explain the large polarization value. The charge distance \( r \) between the localized hot carriers is unambiguously related to the optical dynamics of C-dots. More specifically, the smaller \( r \) hot carrier pairs recombine first as the wave function overlap and the transition matrix decrease with the expanded separation distance, as a result the emission incline to shift to longer wavelength/lower energy as time increase.\(^{182}\). Thus the increasing lifetime as the emission wavelength shift to longer wavelength is expected. Time-resolved PL measurement results are depicted in figure 3.10, for both C-dots A and B, the PL lifetime increase as the emission wavelength increase (sample A from 484.5 nm to 630 nm, sample B from 478 nm to 615 nm) which is consistent with our predictions. We also carried out the time-resolved PL measurement for the C-dots solutions as shown in figure 3.11. Different from the solid films of the C-dot samples, the lifetime decrease as the emission wavelength increase which is similar to the result published by Arjun Sharma\(^{140}\), we attribute the abnormal lifetime tendency to the Brownian rotation\(^{155}\).
We tentatively interpret the origin of C-dots emission. When C-dots excited by monochromatic light, the self-trapped excitons having larger distance $r$ will emit longer wavelength as they undergo relaxation process. As shown above, the relaxation process is suppressed so that the momentum relaxation time is comparable to the PL lifetime. Thus the hot e-e (h-h) and e-h scattering seems not suitable to interpret the relaxation process as it is somehow elastic scattering, which tend to isotropy the momentum distribution but not enormously decrease the energy of hot carriers$^{183}$. Another important scattering process is impurity and defect scattering which is strictly elastic scattering. It has zero contribution to the energy relaxing, instead it can relax the momentum efficiently$^{152}$, which is also contradictory to our results. Therefore the reliable interpretation of the relaxation dynamics is the following picture: photo-excited hot carriers decay as a result of phonon emission, there is no obvious hot carriers scattering and impurity scattering which is different from the band to band emission or band to defect emission. This relaxation dynamics is also anticipated when we consider the self-trapped exciton essence of C-dots emission where the hot carriers are strongly confined such that they do not have enough freedom to accomplish the collision with hot carriers or impurity or defect$^{184}$. 

Figure 3.11 Time-resolved results for the C-dots aqueous solution. (a),(b) for sample A and B aqueous solution respectively.
Based on the distinctive relaxation and recombination properties, the polarization evolution of C-dots emission can be simplified to the model proposed by K. Murayama\textsuperscript{158}. Briefly, during the relaxation process, there are two main channels, one dissipates the energy in the same location which conserve the polarization, while the other channel loss the polarization by hopping which is illuminated by parameter $q$ defined as the ratio between the hopping and the total decay rate. The formula of simplified liner polarization is:

$$P(E_{em}) \approx P_i \left[1 - \left(\frac{E_{ex} - E_{em}}{\hbar \omega}\right)q\right] \quad (2.2)$$

Where $P_i$ is the initial polarization at the instant of excitation, $E_{ex}$ is the excitation energy, $E_{em}$ is the emission energy, $\hbar \omega$ is the phonon energy. Considering the energy difference between the excitation and emission light is over 1 eV, it is reasonable to speculate that the major scattering process is consist of optical phonon scattering which have relative large energy. As proposed by Rusli\textsuperscript{157}, for carbon based materials the typical optical phonon energy is 1500 cm\textsuperscript{-1} or 0.18 eV during the decay process. In order to simplify the calculation, we use the main 0.18 eV energy phonon to approximate $\hbar \omega$, the results are shown figure 3.12 and Table 1. For both C-dots, the polarization have the same value at the instant of excitation (about 0.49) which implies the same emission origin, and the measured polarization (see dot line) match very well with the simplified model (solid line). The degree of polarization of both samples decrease linearly as the emission energy decrease, but the decrease slopes show large difference for C-dots samples A and B. Compared to sample A, the existence of N element in sample B resulting in the faster polarization decay rate, which implies a more rapidly momentum decay process.

For sample B, the introduced N atoms have two effects. First they can act as donors to offer an extra electrons\textsuperscript{103, 185-187} which increase the e-e and e-h scattering processes; second, both the ionized and neutral N atoms can serve as the rigid center to increase the impurity scattering\textsuperscript{183}. And both the hot carriers scattering and impurity scattering will increase the momentum
relaxing rate thus leading to the faster polarization decay. The above explanation is further confirmed by the ratio factor $q$ which describes the hoping probability of hot carriers. The hoping probability of C-dots B ($q=0.08$) is greater than the hoping probability of C-dots A ($q=0.02$) which is consistent with our picture.

![Graph showing polarization vs energy for samples A and B](image)

**Table 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$P_i$</th>
<th>$q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.497</td>
<td>0.02</td>
</tr>
<tr>
<td>B</td>
<td>0.492</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Figure 3.12** Fitting results based on the one-phonon emission approximation model.

We illustrate the electronic structure of C-dots by combining the photo-excitation, relaxation and recombination process, as shown in figure 3.13 (a), where the horizontal axis is the coordinate distance which demonstrate the relative distance of the hot carriers pairs$^{182, 188}$. As the time decays, the larger distance self-trapped excitons will emit smaller energy photons as they undergo the relaxation process. Based on our proposed emission mechanism, the optical phenomena of C-dots can be interpreted. First we investigate the excitation dependent photoluminescence. The PL spectra excited by 325 nm are shown in figure 3.13 (b) (for sample A) and 3.13 (c) (for sample B), which is consistent with the previously reported results$^{98, 189}$. This behavior can be explained satisfactorily by our proposed model. Monochromatic light excites the self-trapped excitons with the energy difference between the two electronic states equal to the excitation photon energy. The suppressed decay process allows for the emission
Figure 3.13 Electronic structure of C-dots (figure (a)). The excitation dependent photoluminescence of sample A (figure (b)) and sample B (figure (c)). Polarization spectra excited by different light, sample A (figure (d)) sample B (figure (e)), blue line: excited by 325 nm, green line: excited by 442 nm. starting from the excitation wavelength. When the excitation light wavelength shifts, it will excite different electronic states of self-trapped excitons, and the suppressed decay process
plays in a similar way as shown above, which leads to the shift of the emission spectrum. Another expected phenomenon is the reduced polarization in the same wavelength range when excited by higher energy laser line. The results are depicted in figure 3.13 (d) (for sample A) and 3.13 (e) (for sample B), the green line is the polarization excited by 442 nm, and the blue line is the polarization excited by 325 nm, it is clear that there are obvious decrease of the polarization in the whole range when excited by 325 laser line. Compared to 442 nm line excitation, the hot carriers excited by 325 nm laser line experience further relaxation process which randomizes the momentum distribution, resulting in the deceased polarization values. Besides the reduced polarization value, the rate of the decrease of polarization also shows some variation when excited by 325 laser line, this may be caused by other scattering processes, like the acoustic scattering\textsuperscript{153} or the existence of the small self-trapped polarons\textsuperscript{182}.

3.3 Conclusion

In summary, we have investigated systematically the optical properties of C-dots by using comprehensive spectroscopic techniques. The degrees of polarization of both PL and excitation as a function of wavelength allow to understand the carrier momentum evolution for different samples, based on which self-trapped excitons are demonstrated to account for the C-dots emission. This assumption is further supported by both excitation power dependent PL, time resolved PL and the PL spectra under high electric field up to1500 V/cm. With the self-trapped exciton model all the optical properties of the C-dots can be explained consistently. Although the fine structure of the self-trapped exciton in C-dots is still unclear, our investigation does provide new physical insight to understand the mechanism of fluorescence from C-dots.
Enlightened by the emission of self-trapped excitons in the SiO$_2$\textsuperscript{179,184}, we speculate that the possible structures of the self-trapped exciton in C-dots are the ruptured C-O bond and/or the peroxo radical bond (one O atom inert into the C-O bond) (see figure 3.14 (b) and 3.14 (c)). Considering the structure of glucose (see figure 3.14 (a)), there exists C-O bond and hydroxyl near the C-O bond, so it is possible to break the C-O bond and/or insert the O atom to the C-O bond during the synthesis process. The localized bond distortion will generate the strong local potential field which resulting in the self-trapping of the hot carriers. For the self-trapped configuration of C-dots, the holes are localized around the O atom (C-O bond configuration) or peroxo radical bond and the electron are trapped by the C atom. Also other self-trapping configurations are possible and the precise structure (such as bond lend, bond angle and the charge distribution, isosurfaces probability distribution of the trapped carriers \textit{etc.}) of self-trapped excitons in C-dots still needs future investigation.
Chapter 4. Dynamic Visualizations in Microfluidics Enabled by Fluorescent Carbon dots

4.1 Introduction

Originated from the demands for biological and molecular analysis and coming into being by technologies of microelectronics, microfluidics have revolutionized application avenues in chemistry, medicine and biology. In microfluidics, the largely increased surface to volume ratio makes the surface tension and viscous stress dominant over inertial effects, rendering a typical laminar flow system, which facilitates fluidic manipulations. The interface becomes extremely interesting, because it can be taken as a moving wall neighboring two phases of desired properties, acting as a membrane. The interface can be used to precisely control the contact time of two distinct chemical or biological components. The interfacial related mechanism, such as the electro-kinetic driven method has been widely adopted in microfluidics, due to the plug shaped rather than the traditional parabolic shaped flow profile, which results in a significantly lower shear stress. The interfacial instability causes breaking off of one fluidic phase, by which the dispersed phase, well known as the droplet is formed. Monodispersed droplets can be formed readily by utilizing T-junction or flow focusing configurations in microfluidics. Particles of micro- and nano- scale have been synthesized in microchannels by solidification of the generated droplets. Droplet based systems offer a variety of advantages: low reagent consumption, high throughput, short duration for analysis and high sensitivity.

Besides the fascinating fluid physics at reduced scales, promising applications of microfluidic platform in biologics, chemical reaction, drug delivery, bacterial detection, synthesis, and energy harvest, have inspired extensive fundamental investigations on precise control of solute transport such as mixing, interface control, droplet and complex droplet formation mechanism, etc. Crucial to such investigations is
the dynamic visualization of fluid flow in a microfluidic circuit. To fit applications in critical fields such as biologics studies, medicine synthesis and drug delivery, fluorescent markers necessary for the visualization must have combined merits of photostability, chemical inertness, high imaging intensity, biocompatibility, environmental friendliness and above all, low cost. Currently, experimental technologies in flow field visualization exclusively rely on fluorescent dyes or dye based seeding particles\textsuperscript{224}. Traditionally chosen fluorescent dyes were not satisfactory markers because they suffered from either poor biocompatibility or extremely high cost. Moreover, the relatively low emission intensity of the fluorescent dyes also hinders the exploration of high speed dynamics, such as formation of nano liter droplets.

In quest of desired marker materials for microfluidics, our attention has been attracted to fluorescent carbon dots (C-dots) because of their naturally endowed biocompatibility, environmental friendliness and cost affordability. Indeed, considering the favorable characteristics of photoluminescence (PL) and flexibility of surface function groups, zero dimensional carbon based materials perfectly meet all the requirements for microfluidics, especially in applications of biology, chemical synthesis and drug delivery, etc. In fact, fluorescent C-dots have been attracting extensive interest in the past decade due to their chemical inertness\textsuperscript{225}, high photostability\textsuperscript{134}, low toxicity\textsuperscript{133, 226}, and low cost\textsuperscript{227, 228} rendering themselves great potentials in the fields such as photocatalysis\textsuperscript{229}, illumination\textsuperscript{133, 230, 231}, energy harvesting\textsuperscript{232}, light emitters\textsuperscript{233, 234}, especially bioimaging and drug delivery\textsuperscript{133, 230, 235}. Compared to traditional quantum dots which involve heavy metals\textsuperscript{118, 236}, C-dots are biocompatible and environmental friendly in terms of both the material composition and their synthesis procedures\textsuperscript{237}.

However, to the best of our knowledge, there has been no report on employing C-dots for microfluidic visualizations. We strategically introduce fluorescent C-dots into microfluidics as tracer markers and exploit their good merits. Our attempt is clearly validated by consistent
investigation on selected important interface phenomena in microfluidics, such as mixing induced by electrohydrodynamic instability, hydrodynamically controlled interfacial cladding, and droplet & complex droplet formations. Furthermore, we succeed in quantitatively measuring flow fields at microscale by means of synthesized C-dots micro particles (CDsP), featuring excellent imaging intensity, good biocompatibility and a remarkably lower price relative to the traditional dye-based markers. Our results demonstrate that fluorescent carbon dots can serve as an advantageous whereas affordable material for visualization in microfluidics which may not only find plenty of applications in biology, chemical synthesis and medicine industry but also enable quantitative studies of high speed dynamics in fluids at microscale.

4.2 Results

4.2.1 Optical characteristics of C-dots

Fluorescent C-dots were synthesized from glucose and urea (both from Sigma Aldrich) by adopting microwave assisted method\textsuperscript{238} (see Chapter 2.2.2). The TEM image of the synthesized C-dots in figure 4.1 (a) shows the sizes of the C-dots are less than 10 nm which agrees well with the published results\textsuperscript{134, 239}. The spectra of optical absorption and photoluminescence (PL)
under excitation of 442 nm are plotted in figure 4.2 (a) for C-dots aqueous solution. The sample displays a broad absorption range from UV to visible, which makes versatile the excitation light source for microfluidic visualizations. As compared to largely used traditional dyes in microfluidics or fluorescent protein in biological science, C-dots show strong fluorescence with unique excitation dependent photoluminescence (figure 4.1 (b)).

![Figure 4.2](image)

**Figure 4.2** Optical properties of C-dots in DI water. (a) Spectra of photoluminescence (PL) and absorption of the C-dots, (b) C-dots sample excited by a 442 nm laser.

The luminescence mechanism for C-dots was discussed in Chapter 3. We notice that under the excitation of 442 nm line from a usual He-Cd laser, the C-dots demonstrate strong green emission as shown in figure 4.2 (b). This color is most sensitive for human’s eyes and many photodetectors, thus very suitable for microfluidic visualizations. It is worth pointing out that, unlike the commonly adopted fluorescent dyes in biological environment whose widespread applications are hindered by the high cost, the cost of our C-dots is very low because they can be obtained from cheap and reproducible materials such as glucose, citric acid, etc.\(^{134}\). Moreover, the synthesizing procedure of C-dots has distinct advantages of simple fabrication, high throughput and no contaminations to the environment\(^{228,240}\) thus C-dots can be an ideal replacement to the currently utilized dyes. In the fields of microfluidics, the flexibility in
surface function group makes C-dots adaptable in both oil based and aqueous systems\textsuperscript{231}. Besides the advantages mentioned above, in the practical applications, chemical inertness, photostability and toxicity are also critical. We have verified C-dots’ chemical inertness by measuring photoluminescence spectra of C-dots synthesized one year ago, which is illustrated in Figure 4.3 (a) and 4.3 (b). The previously synthesized C-dots still show bright green emissions and the spectra are nearly the same with that of the freshly synthesized sample under

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.3.png}
\caption{(a), (b) Chemical inertness of the synthesized C-dots solution. (a) PL of the C-dots solution, (b) image of C-dots solution excited by a 442 nm laser. The C-dots sample was synthesized on 07 December, 2015 and the test was carried out on 07 December, 2016. (c) Photostability of the C-dots solution by plotting the integrated intensity of the PL spectra for 3 hours with the frequency of 6 times per hour.}
\end{figure}
442 nm excitation. We also carried out the photostability exploration of C-dots by 3 hours’ continuous laser excitation at the power density of 3.96 W/cm$^2$. The results show that both emission intensity and the line shape of the spectra are stable, which proves the great photostability of the synthesized C-dots (figure 4.3 (c)). Moreover, we verified the biocompatibility and low toxicity by the cytotoxicity experiment. We measured the survivability of 293T and HeLa cells in solution containing a high concentration C-dots by means of cell counting kit-8 (CCK-8). The concentration of C-dots is tens of times higher than the concentration adopted in practical applications. The good survivability shown in figure 4.4 proves the excellent biocompatibility and low toxicity of C-dots. All of these pave the way toward widespread applications of fluorescent C-dots in microfluidic environment.

![Graph](image)

**Figure 4.4** Cell survivability of 293T and HeLa after culturing with C-dots for 24 hours. The measurement was carried out by using of CCK-8 assay.

### 4.2.2 Visualizations of mixing via electrohydrodynamic instability

Mixing is a fundamental phenomenon with wide applications including food industries, medical treatment, chemical reaction, drug delivery, etc. Methods such as turbulence flow adopted for mixing at macroscale may not be feasible at microscale due to the small Reynolds number (Re) where the nonlinear inertia effect becomes negligible resulting in the difficulties
in creating turbulences. Consequently, the required fast and efficient mixing at microscale is challenging. Both the passive and active mixing methods are proposed at microscale, among which electrohydrodynamic instability induced mixing is the promising one. We study this critical interface phenomenon in microfluidics by utilizing C-dots as a fluorescent marker. Two aqueous fluids, C-dots based solution and mixture of glycerol & deionized water (DI water) were interfaced in the microchannel and flowed in parallel under the tangential electric field as shown in figure 4.5 (a). It can be clearly seen that the interface was initially flat upon hydrodynamical driving \((t=0 \text{ s}, \text{figure 4.5 (b)})\) and a raptured interface emerged between the two fluids shortly after applying the electric field \((t=0.6 \text{ s}, \text{figure 4.5 (b)})\), followed by a further distortion until the interface was broken and a complete mixing was achieved \((t=2.0 \text{ s} \& t=3.76 \text{ s}, \text{figure 4.5 (b)})\) respectively. The complete dynamic process can be viewed in a video provided in SI, movie S 4.1. The mixing procedure was imaged by using photoluminescence of C-dots (figure 4.2 (a)) and the mixing efficiency was quantitatively calculated by means of image processing using a self-programmed matlab code. The resultant mixing efficiency is defined as the discrepancy between value 1 and the non-dimensionalized standard derivation of the image intensity. The trend of mixing by applying tangential electric field (see figure 4.5 (c)) indicates an oscillating but growing mixing efficiency. Moreover, we can only obtain a partial mixing under a relatively weak electric field, whereas a strong field breaks the “boundary” between the two fluids and mixes them thoroughly which is shown in figure 4.5 (d). The electrohydrodynamic instability induced mixing is balanced by the pressure gradient \(-\nabla p\) \((p: \text{pressure})\), viscous dissipation \(\mu \nabla^2 u\) \((\mu: \text{dynamic viscosity of the fluid, } u: \text{velocity field})\), and electrical field induced body force \(-\rho_e \nabla \Phi\) governed by the conservation law of the ion species in each fluid \((\rho_e: \text{electric charge density, and } \Phi: \text{electrical potential})\). Balanced by all the forces, a threshold of the electric field exists to induce a fully mixing which is caused
by the conductivity gradient orthogonal to the flow direction. Electric field below than the threshold will cause an oscillating pattern without a complete mixing while the electric field larger than the threshold will achieve a rapid and through mixing at microscale. The mixing dynamics are mostly decided by the electroviscous and diffusion time scale. The electroviscous time scale is derived from the balance of viscous force with the electric force 

\[ \tau_{ev} = \frac{W}{U_{ev}} = \frac{W}{(\varepsilon E^2 \mu W^2)} = \mu (\varepsilon E^2) \],

where \( W \) denotes the width of the microchannel, \( \varepsilon \) is the electric permittivity of the specific fluid, \( U_{ev} \) depicts the electroviscous velocity scale and \( E \) represents the electric field strength \(^{241}\). Another time scale for evaluation of mixing through diffusion is defined as \( \tau_d = \frac{W^2}{D} \), where \( D \) is the diffusivity of the fluid. To evaluate the mixing performance caused by the electric field, we can compare the two time scale

---

**Figure 4.5** Electrohydrodynamic instability induced interfacial mixing characterized by C-dots. (a) schematics of the proposed interfacial mixing method, (b) mixing evolution at applied electric field strength of 136.36 KV/m, (c) mixing procedure characterized by mixing efficiency, (b) maximum mixing efficiency achieved by various electric field strengths. Scale bars represent 100 µm. The electric field is applied by inserting two Pt wires into the inlet of C-dots and the outlet acting as the anode and cathode respectively.
\[
\tau / \tau_e = \frac{\varepsilon W^2}{\mu D} E^2
\]
and see the ratio is proportional to \( E^2 \), which explains the enhancing mixing efficiency with an increasing electric field strength \( E \) as shown figure 4.5 (d). In this exploration, the emission intensity of C-dots was not influenced by the high strength electric field and the raptured interface was captured by employing C-dots successfully. Thus, C-dots present themselves to be an excellent choice for a tracing marker under high electric field strength.

4.2.3 Visualizations of Interface cladding and control of interfacial positions hydrodynamically

Microfluidics is known for the benefits of the multi-step processing integrated in one single chip (“lab on a chip”). In the applications of chemical reactions and drug delivery, reagents can be tested in various conditions by means of flowing through distinct flow branches. Therefore, the function of flow switching becomes critical. We propose a flow cladding system as shown in figure 4.6 (a) to accomplish such a function. This system is achieved by two cladding phases (\( Q_{L1} \) and \( Q_{L2} \)) at the two sides and one core phase (\( Q_c \)) in the center. The flow switching of the core phase by controlling the interfaces can be realized by tuning the flow rate ratios (\( Q_{L1} / Q_c / Q_{L2} \)) among the two cladding phases and the core phase. The flow switching is governed by the Navier-Stokes equations. Under the conditions of static state, fully developed flow field, Newtonian and small Re number flow, the governing equations can be simplified as

\[
\nabla p = \mu \nabla^2 \mathbf{u}, \quad \text{where} \quad p \text{ is the hydrodynamic pressure, } \mu \text{ and } \mathbf{u}, \text{represent the dynamic viscosity and velocity field of the each fluid, specifically in our case, two cladding phases (} Q_{L1} \text{ and } Q_{L2} \text{) and one core phase involving C-dots (} Q_c \text{). With proper boundary conditions which are } \mathbf{u}=0 \text{ at wall, } \mathbf{u} = \mathbf{u}_{i+1} \text{ at the interfaces, and the constant flow rates } Q_i = \iint_{S_i} \mathbf{u}_i(x, y) \, dx \, dy, \]
the governing equations can be solved numerically so as to the position of the core phase. The core-cladding process is determined by the balance of hydrodynamic pressure (controlled by $Q_{L1}$, $Q_{C}$, and $Q_{L2}$) and viscous stress (tuned by $\mu_{L1}$, $\mu_{C}$, and $\mu_{L2}$). By tuning the parameters, $Q_{L1}$, $Q_{C}$, $Q_{L2}$, $\mu_{L1}$, $\mu_{C}$, and $\mu_{L2}$, the core phase can be switched to different branches hydrodynamically for intended processes. Fluorescent imaging of the core phase visualized by

![Diagram](image)

**Figure 4.6** Flow switching by a core-cladding configuration. (a) Schemes of the core-cladding configuration, (b) positions of the core phase (C-dots) under various flow rate ratios ($Q_{L1}/Q_{L2}$), (c) positional dependency of the core phase on the flow rate ratio between the two cladding phases, where the location was non dimensionalized by the width of the microchannel. Scalar bars indicate 50 µm.

C-dots were recorded and shown in figure 4.6 (b). The central position of the core phase was measured by tracking the two interfaces between the core phase $Q_{C}$ and the two cladding phases $Q_{L1}$, $Q_{L2}$. The position of the core phase shifts from left to right by increasing the flow
rate ratio between the cladding phase 1 and 2 \( \frac{Q_{c1}}{Q_{c2}} \) with the flow rate of core phase constant \( Q_c \). The dependence of the core position on the flow rate ratio of the two cladding phases was presented in figure 4.6 (c). By proper control of the flow rate ratio between the two cladding phases \( \frac{Q_{c1}}{Q_{c2}} \), we can achieve the position shift up to 50% of the whole microchannel.

It should be highlighted that good photostability is necessary for a tracer material to address problems at static or pseudo static state. The great photostability of C-dots adopted here is crucial for exploring the quantitative correlation between the position and flow conditions.

### 4.2.4 Visualizations of Droplets and complex droplets

**Droplet formations**

In recent years, enormous interests have been attracted to the formation of monodispersed droplet in microchannels for applications of microreaction technology, biomedical industries.\(^{196, 206, 242, 243}\). Droplets with large surface to volume ratio reduce the reaction time significantly. Uniform droplets of nano liter or even pico liter size can be prepared using a flow focusing or T-junction microfluidic configuration. In both configurations, the fluid used to form droplets is normally termed as the dispersed phase \( Q_D \), with the other fluid which is not broken into droplets termed as the continuous phase \( Q_c \). Here we straightforwardly observe the formation of droplets by adopting C-dots based aqueous solution as the dispersed phase, and the mineral oil based fluid as the continuous phase. Three typical formation regimes in a flow focusing configuration are presented: squeezing, dripping and jetting, as shown in figure 4.7 (a). Droplet diameters were measured through image processing and plotted as a function of capillary numbers \( Ca \) as illustrated in figure 4.7 (b). The capillary number \( Ca \) is the ratio between two dominating effects during droplet formation: the viscous effect and interfacial tension,
defined as \( Ca = \frac{\mu U}{\gamma} \), where \( \mu \) is the dynamic viscosity of the continuous phase, \( U \) is the characteristic velocity of the continuous phase and \( \gamma \) depicts the interfacial tension between the dispersed and continuous phases. An inverse growing of droplet diameter with \( Ca \) was obtained in figure 4.7 (b). This is because the higher interfacial shear reduces the size of the droplet. The droplet formation regime shifts from squeezing to dripping and finally reaches jetting with the increasing of \( Ca \). Each formation regime has its unique range of droplet sizes. A quantitative scaling of the droplet diameter as the function of \( Ca \) in the form of \( D = \phi(Ca)^\theta \) can be proposed, where \( D \) is the droplet diameter, \( \phi, \theta \) are the fitting parameters obtained from the experiments. The scaling law indicates that the droplet formation is dominated by two leading factors: viscous stress and surface tension. The scaling law and the curve in figure 4.7 (b) act as the guideline on the flow conditions that can generate droplets of desired sizes.

**Figure 4.7** Applications of C-dots in droplets and complex droplets. (a) Droplet formations in three typical regimes: squeezing, dripping and jetting, (b) correlations between capillary number and droplet diameter. Scalar bars represent 50 µm.

**Double component droplets**

We employed two fluids, C-dots aqueous solution and mixture of glycerol as the dispersed phase, and the mineral oil as the continuous phase for generating the double component droplet as depicted in figure 4.8 (a). The appearance of double or multi component droplet origins from the requirements in the fields of chemical reaction, bio-medical analysis, catalysis and drug
delivery, which normally involve more than one component. In all the above mentioned applications, not only various portions of distinct components should be tested, but also the controlling accuracy should be guaranteed. The flow is typically laminar due to the small Re number and the flow rate as low as nanoliter per hour can be accessed with the help of modern syringe pump. With refined fluidic control and the droplet formation configuration at microscale as shown in figure 4.8 (a), we can achieve an accurate control of each component effortlessly by tuning flow rates of specific samples. By increasing the flow rate ratio of C-dots over glycerol from 10:35 to 30:15, the bright portion of the droplet becomes larger indicating the increasing amount of C-dots over glycerol solution (see SI, movie S4.2). Furthermore, the mixing within a double or multi component droplet needs to be investigated, since a full mixing is critical for chemical reaction or catalysis. In this situation, an interface is formed and the diffusion takes the leading effect on the mixing procedure. The implementation of C-dots allows us to study the mixing dynamics so that optimum conditions, such as the flow field, can be chosen to encourage a rapid mixing.

**Merging of the double component droplet under a DC electric field**

In some conditions, selective locations or specific droplets for reactions are required. In practical cases, the controlling flexibilities determine the scope of applications. As a consequence, we propose another approach, droplets containing different reagents (may be single or multi component), are generated and merged selectively via the applied electric field. As a result, a systematic exploration can be carried out by merging two or even more selected droplets, offering a controlled chemical reaction. We illustrate the merging dynamics by applying a DC electric field (whose direction is normal to the flow direction) using C-dots to label different types of droplets. Two identical double component droplets were merged and the dynamics were present in figure 4.8 (b) and the process can also be seen in SI, movie S 4.3. The interface was deformed shortly after applying the electric field (t=8 ms) so that the two
boundaries of the neighboring droplets was close to each other. Due to electric field induced Maxwell stress, the surface tension was reduced and merging occurs at \( t = 15 \text{ ms} \). The merging of the droplet is a balance of viscous force, interfacial tension, and electric body force. When electric field \( E \) induced electrical stress \( \sigma_E \propto \varepsilon E^2 \) is enough to over the capillary pressure \( p_c \propto \gamma / R \), the shape of the droplet is deformed, where \( \varepsilon \) is the electric permittivity, \( \gamma \) and \( R \) denote the interfacial tension and radius of the droplet respectively\(^{244}\). The dipole-dipole interaction becomes stronger when the space between the two neighboring droplets turns smaller resulting in the occurrence of the merging. The spacing between the two droplets, fraction of each component in the droplet and the concentration of the glycerol within the glycerol mixture hold major influences to the merging. Detailed operation map for the merging can be obtained via a systematic investigation utilizing C-dots. Limited by the scope of this article, we do not intend to report the details here. The merging system has great potentials in high throughput chemical reactions, catalysis and screening bioassays\(^{245}\).

**Figure 4.8** (a) Various double component droplets with C-dots and mixture of glycerol & DI water (gly. mix.) as the dispersed phase (b) merging of such double components droplet under a normally applied DC electric field at the strength of 9 MV/m. Scalar bars represent 50 µm.
Double emulsions

Double emulsions, the unique "core-shell" like structure endow various distinctive applications in sensing, drug releasing, and capsulization\textsuperscript{221, 222}. For biosensing, sensing materials with poor biocompatibility may find their niches when surrounded by the outer biocompatible droplet. A controlled drug releasing at a low rate become feasible if the functional drug is enveloped by an outer layer which controls the diffusion of the inner droplet to the environment. The widely adopted capsulization in medical industries was favored by the double emulsion resulting from the accurate and easy control of the "core-shell" like structure.

Double emulsion can also be used to fabricate hollow particles with perfect sphere shape. In this sense, the entire formation process is of intense interest for research. We adopted mineral oil as the inner droplet, C-dots based aqueous solution as the outer droplet and an oil based solution as the continuous phase (figure 4.9 (a)). Firstly the inner oil droplet was generated by the shearing of the C-dots based aqueous solution in the “Y” shape junction, then the inner droplet along with the C-dots flowed into the flow focusing junction where the double emulsion was formed by shearing of the oil based continuous phase (the microchannel configuration can be seen in Chapter 4.5.5). The dynamics in the flow focusing junction is shown in figure 4.9 (b) starting from $t=0$ ms to $t=56$ ms (movie is available in SI, movie S 4.4). The formation mechanism is similar to the aforementioned droplet formation, but the difference is to create the two junctions of distinct wettability (hydrophilic for the “Y” shape junction and hydrophobic for the flow focusing junction, details can be seen in SI, section “Microchannels utilized in the experiments”). Moreover, the double emulsion can extend to tribal or even multi emulsions, and the inner droplet can be one or multi. Break up and merging phenomena are expected by applying external fields (e.g. electric field). C-dots offer the opportunity for studying the phenomena and investigating the conditions for generating such complex droplets.
4.2.5 Flow field tracking by fluorescent C-dots micro particles (CDsP)

By means of C-dots, we succeed in visualizing the interfaces in both static and dynamic situations. To further study the details and understand the fluidic physics, a comprehensive and quantitative measurement of the flow field is essential. The most common method adopted to measure flow field is the particle image velocimetry (PIV) which uses a thin layer laser sheet to illuminate particles following the flow field. However for flows at microscale, the laser illuminated measurement plane is usually thicker than the desired one which induces extra noise into the measurement. The fluorescent seeding particles other than the normal particles along with small optical focusing depth were employed at microscale to enhance the signal to noise ratio, which becomes the well-known micro-PIV technology. To make use of the advantages of C-dots such as high imaging intensity, good biocompatibility, low cost, etc., for the first time we introduce the fluorescent C-dots micro particle (CDsP) as the seeding particle into flow field measurement.

We synthesized the CDsPs by stir-mixing the porous polystyrene micro particle. Polystyrene based materials are well known for their nontoxicity and low cost, which ensure both the low cost and excellent biocompatibility of our CDsPs. Detailed procedure of the synthesis can be seen in Chapter 2.2.2. The SEM image shown in figure 4.10 (a) of the CDsP
illustrates the porous structure and the diameter is around 3 µm. Figure 4.10 (b) depicts the PL spectra of the pure particle and the CDs P. When excited by 442 line, there is no obvious emission from the pure particle. Instead, CDs P show green emission similar to the C-dots based aqueous solution, which indicates CDs P can be used in quantitative measurements of flow field. The fluorescent image of droplet formation with CDs P in the dispersed phase is shown in figure 4.10 (c) and the movie can be accessed in SI, movie S 4.5. We successfully achieve a quantitative measurement of the flow field by correlating the CDs P illuminated images as shown in figure 4.10 (d). An enlarged view at the tip of the droplet is obtained by subtracting the mean velocity of CDs P, and two flow recirculation patterns are presented. In order to verify the measured velocity field, we carried out the simulation of droplet formation as shown in figure 4.10 (e) (details can be seen in Chapter 4.5.6). Considering the whole dynamics of the droplet formation, and the flow field distribution including the two flow recirculation patterns at the tip of the droplet, the micro-PIV measurement obtained via employing CDs P gives reasonable agreement with the simulation, although there is some discrepancy in terms of velocity scale, which is majorly caused by simplifying droplet formation into a 2D condition during the simulation. Nonetheless, we have shown the validity of utilizing CDs P in flow field measurement at microscale, which manifest the promising applications of CDs P in the quantitative measurement for microfluidic physics.
Figure 4.10 Flow field measurement in microfluidics by employing CDsPs. (a) SEM image of the synthesis CDsP, (b) PL spectra of the porous polystyrene micro particles before and after synthesis, (c) the fluorescent image of droplet formation with CDsPs distributed in dispersed phase, (d) typical flow field of droplet formation obtained by micro-PIV correlation of the recorded imaged illuminated by CDsPs, (e) simulated flow fields of droplet formation. Scale bars represent 50 µm if not specifically indicated. Both the flow measurement and simulation were carried out at the flow rates of CDsPs aqueous solution versus mineral oil based solution =20 : 100 μL/h.

4.3 Discussion

Beside the chemical inertness and photostability, practical applications of parallel flow and droplets/complex droplets ask for different requirements from fluorescent materials. The parallel flows (figure 4.5 and figure 4.6) are static or pseudo static cases in which a relative low imaging speed is acceptable. However the whole formation procedure of droplets or complex droplets is within tens of ms, which is a typical dynamic problem. A relatively strong emission of the fluorescent material is imperative for capturing such dynamics. Fortunately, we are able to achieve acceptable image intensity within exposure time of 400 μs (figure 4.7 & 4.8.). Due to the high imaging intensity enabled by C-dots, we are able to capture images up to
2500 frames per second by using one normal continuous He-Cd laser with power of 67 mW. Traditionally, we can hardly visualize different fluidic systems (aqueous or oil, organic or inorganic) by using one type of dye. C-dots show great advantages of flexibility in different fluidic systems as it is easy to synthesize aqueous or oil, organic or inorganic based C-dots. Therefore C-dots find themselves a niche for studying complex droplets, especially multi emulsions.

CDsPs enjoy the advantages of great imaging intensity benefiting from highly fluorescent C-dots. Excited by a common He-Cd laser source with the excitation power of only 67 mW, we obtain the fluorescent images at the exposure time of 2 ms, which can support up to 500 frames per second. Armed with additional advantages including chemical inertness, photostability, and flexibility to adapt to various fluidic systems, the CDsPs are expected to show great potentials for quantitative characterization of dynamics in microfluidics. Moreover, as a result of the low cost of C-dots and convenience of the synthesis, we are able to synthesize the CDsPs at only one-tenth the price of the commercial fluorescent particles, not to mention the great biocompatibility over that of the conventional dye based seeding particles.

4.4 Conclusions

The implementations of C-dots in microfluidic environment for the applications of mixing, fluidic cladding, droplets and complex droplets have shown superior potentials. Due to the perfect biocompatibility, low toxicity and environmental friendliness both in the synthesis and the production, C-dots prove themselves an ideal replacement of conventionally utilized dyes. The low cost and easiness in synthesis make the C-dots ready for commercial production, which might lead to a revolutionary change in the industries.

For the first time, we proposed and succeeded in employing CDsPs for quantitative measurements of flow fields. By using CDsPs we are able to realize imaging at a high speed
under a normal laser source excitation, which enable the studies on real time flow field dynamics. Furthermore, we were able to cut off the 90% cost of traditional fluorescent seeding particles by introducing CDsPs, making the dynamics studies truly affordable. The inborn nature of high fluorescence intensity, perfect biocompatibility and low cost make CDsPs an ideal replacement for conventional fluorescent particles. We anticipate that fluorescent C-dots and CDsPs would be a game-changer for microfluidic visualizations and will bring about enormous impact in the development of microfluidics.

4.5 Experiments and simulations:

4.5.1 Chemical inertness of C-dots
We verified the chemical inertness of the C-dots by measuring the PL spectrum of a C-dots sample one year after the synthesis. Figure 4.3 (a) shows the PL spectra which are almost identical to that measured from a freshly synthesized one (figure 4.2 (a)). Under a normal 442 nm He-Cd laser excitation, it gives strong green emission as shown in figure 4.3 (b). The results indicate that the chemical properties of C-dots exhibited no obvious change over a long period of time, which verifies the chemical inertness of the C-dots.

4.5.2 Photostability of C-dots
We did one set of experiments to prove the photostability of the synthesized C-dots solution. The C-dots solution is continuously excited continuously by the 442 nm He-Cd CW laser under the power density of 3.96 W/cm² for three hours. We did the PL spectra measurement for every 10 minutes and integrate the intensity over the range of 450nm to 700 nm. Both the integrated intensity (figure 4.3 (c)) and the lineshape (in the subfigure) are the same, which indicate the great photostability of our C-dots solution
4.5.3 CCK-8 assay for the cytotoxicity

Cell counting kit-8 (CCK-8) assay is a colorimetric assay for quantitative determination of cell viability in cell proliferation and cytotoxicity assays. To verify the biocompatibility and low toxicity of C-dots, the in vitro tests were carried out on two kinds of cells, 293T and HeLa, by using CCK-8 assay. 100 µL of cells at the concentration of $10^5$ cells/ml was seeded in a 96-well plate. Four concentrations of C-dots 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml were chosen for the exploring the viability of the two types of cells. After the cells were cultured in a 5% CO₂ incubator at 37 °C for 24 hours, the C-dots of different concentrations were added into the sample and cultured for another 24 hours. And then the sample was treated with 10 µL CCK-8 and cultured for 4 hours. Four replicate wells were prepared for each control and tested concentrations. In the end, the optical density of each sample was recorded by a microplate reader at a wavelength of 450 nm for analysis.

As shown in figure 4.4, the influence to the viability of both 293T and HeLa after 24 hours’ incubation with C-dots was marginal even the concentration of C-dots is much higher than the required one in really applications. The high viability of the two cells proves the good biocompatibility and low toxicity of C-dots.

4.5.4 Fluidic sample prepare for the experiments

In the mixing experiment (figure 4.5), two fluids, C-dots solution and 30% (by weight) mixture of glycerin (Sigma-Aldrich) with DI water were interfaced, while in the cladding experiment (figure 4.6), C-dots acted as the core phase with 10% of glycerin mixture acting as the cladding phase. During the droplet formation (figure 4.7 (a)), an oil/ C-dots configuration was utilized. The oil was mineral oil (M5904 from Sigma Aldrich) mixed with 5% Span 80 (Sigma Aldrich). We used C-dots, 30% glycerin mixture and mineral oil with 5% Span 80 as the two dispersed phase and one continuous phase respectively (figure 4.8 (a) & (b)). For the double emulsion experiment, pure mineral oil formed the inner droplet, with 65% of glycerin mixed
with C-dots as the outer droplet and the mineral oil with 5% Span 80 as the continuous phase (figure 4.9 (a) & (b)).

4.5.5 Microchannels utilized in the experiments

![Figure 4.11](image)

**Figure 4.11** Schematics of the microchannels employed for interfaces formed by two types of liquid phases. (a) The microchannel used for mixing via electrohydrodynamic instability (figure 4.5), (b) microchannel utilized for interface cladding (figure 4.6).

The microchannel employed for the investigation of mixing via electrohydrodynamic instability is shown in figure 4.11 (a), which is a microchannel cable of introducing two fluids to form an interface with the width of 300 µm. The electric field is applied by inserting two platinum wires (from Sigma Aldrich) into the inlet and outlet of the microchannel respectively.

Figure 4.11 (b) demonstrates the microchannel utilized for interface cladding hydrodynamically with a characteristic length scale being 100 µm. The microchannel used the flow focusing configuration with the central fluid being the C-dots while the other two being the cladding phases.
Figure 4.12 Microchannel utilized for droplet generations and flow field measurement. (a) Schematics of the microchannel for droplet formation and double component droplets, (b) photo of the corresponding microchannel, (c) schematics of the microchannel for generation of double emulsions, (d) photo of the microchannel for formation of double emulsions, (e) schematics of the microchannel for flow field measurement via utilizing CDsPs. The red ink filled into the microchannels in (b) (d) was for better illustrations.
Figure 4.12 (a) & (b) show the schematics and photo of the microchannel adopted for droplet generation (figure 4.7 (a)), formation of double compound droplet (figure 4.8 (a)), and merging of double compound droplet (figure 4.8 (b)). A flow focusing configuration with channel width of 100 µm was employed. A 50 µm orifice was designed to stimulate the break off of droplets. Four electrodes were fabricated by melting indium alloy (Indium Corporation of America) into the electrode channel, which was then connected with the high voltage supply system via electric wires.

A “Y” shape junction and a flow focusing junction were adopted for exploring the formation of double emulsions, as shown in figure 4.12 (c), (d). An UV initialized polymerization was chosen for the surface modification to facilitate the formation of oil based droplet.

A “Y” shape microchannel with the width of the inlet at 100 µm and outlet at 300 µm is employed for the flow field measurement by introducing CDsPs, as schemed in figure 4.12 (e).

4.5.6 Numerical simulations on droplet formations

We conducted the numerical simulations for droplet formations by adopting level-set for interface tracking which requires the mesh of high quality. The mesh was constructed by mapping and connecting small regions divided manually. The resulting mesh can be seen in figure 4.13 (a) & (b). The mesh with good quality helped to address the convergence issue and enhance the computational efficiency. The interface between the dispersed and continuous phases was successfully tracked as depicted in the phase plot of the droplet formation (figure 4.13 (c)) which is in good agreement with the experiment observations.
Figure 4.13 Mesh and phase plot of the simulation for droplet formations. (a) Overall view of the generated mesh, (b) enlarged view of the mesh at the junction, (c) phase plot during the droplet formation process
Chapter 5. Enabling Quantitative Investigation of Fast and Complex Flow Field in Microfluidics by Self-assembled CsPbBr$_3$ Perovskite Flow Tracers

5.1 Introduction

The modern technologies have made possible the precise and sophisticated manipulations of fluidics at microscale (also termed as microfluidics)$^{190, 192, 193}$. Due to the niche scale matching the features of cells $^{246, 247}$ as well as other merits such as low sample consumption $^{191, 248, 249}$, high surface to volume ratio $^{212, 250}$, “lab on a chip” integration $^{207, 251-254}$, etc., microfluidics has been attracting more and more attention in chemical reaction $^{4, 32, 212, 255, 256}$, biological detection $^{246, 247, 257}$, material synthesis $^{258, 259}$ and so on. Underpinning these applications, quantitative flow field measurement in microfluidics is essential for innovative design of microfluidic circuits, and is also imperative for fundamental studies on chemical reaction and fluid mechanics at microscale. Currently the most powerful technique for quantitative flow measurement is the micro-scale particle image velocimetry ($\mu$PIV) which measures the vector field of the flow (both the direction and magnitude) by correlating images of the seeding particles inside. Critical to $\mu$PIV are pertinent fluorescent seeding particles which serve as flow tracers inside an interrogated fluid at microscale $^{224}$. Unfortunately, current fluorescent tracer particles suffer from low emission intensity as most of them are synthesized by coating fluorescent materials (dyes or semiconductor quantum dots) onto the surface of non-fluorescent spheres, which limits the imaging speed and shows vulnerability in capturing fast dynamics.

The droplet based microfluidics provide versatile tools for chemical reaction, cell based analysis, drug delivery, etc $^{196, 260, 261}$. They enjoy distinct high processing efficiency up to a few thousands of droplets per second. To capture details of such dynamics, an ultra-fast fluorescent imaging speed in the order of tens of thousands per second is required. Currently, commercially available high speed micro-PIV systems rely on high frequency, high power, multi-cavity, pulse lasers to achieve a capturing speed of a few thousand frames per second.
while suffering from high cost, high system complexity and the unwanted heating effect. Nonetheless, the capturing speed is still inadequate. Currently adopted strategy for high capturing speed has been focusing more on the illuminating laser, albeit the low emission intensity of the synthesized tracer particle is the major challenge for the measurement technique at microscale. Here, we show that we can overcome this challenge by employing novel luminescent tracer particles at micron scale made from all-inorganic halide perovskite quantum dots (QDs).

Since year 2015, all-inorganic cesium lead halide perovskite CsPbX₃ (X=Cl, Br, and I) have been attracting increasing attentions because of their unique properties such as the high photoluminescence quantum yield (PLQY), relatively improved thermal and moisture stabilities, tunable emission wavelength, long carrier time and narrow emission peak. They are showing great potentials in solution-processable optoelectric devices, for instances, solar cells, light emitting diode (LED) and lasers. The merits of inorganic perovskite QDs render themselves the potential in high speed flow field measurement at microscale. However, there are two important issues that hinder the applications of halide perovskite QDs in flow measurement at microscale. First, perovskite QDs cannot be directly used in flow field measurement because the diameter of halide perovskite QDs usually lies in several to tens of nanometers while the sizes of particle used in μPIV should be in the order of micron or submicron to avoid random blinking, Brownian motion and weak emission signal from a single nanoparticle. Second, the inorganic perovskite QDs are highly unstable once encountered an aqueous phase. In order to address these two issues, we implemented a new approach of self-assembling the cesium lead halide perovskite CsPbBr₃ QDs into micron scale particles in mineral oil. The synthesized particles are protected by the oil phase and thus can be applied in aqueous based phases. Different from the commercially available fluorescent particles synthesized by a layer of coating on the surface,
our self-assembled particles are completely composed of the perovskite QDs, thus they are brighter and can achieve a much higher imaging speed. Moreover, the cost of our approach is much lower than the commonly used fluorescent seeding particle method, as the coating process is not required.

Taking advantage of the high photoluminescence quantum yield (PLQY) of cesium lead halide perovskite CsPbBr$_3$ QDs and the novel assembling approach, we achieve ultra-fast, quantitative measurement of flow fields up to 10,000 frames per second in a single CW laser system with satisfactory signal to noise ratio. To the best of our knowledge, this is the fastest quantitative measurement speed ever reported in the field of microfluidics. We further verify our approach by successfully showing a few case studies of fast and complex flow field in microfluidics including single/multi-layer flow, microdroplets and complex droplets. Our finding not only allows for more precise studies of dynamic flow fields at microscale by offering higher measurement speed, but also enriches the application scope for all-inorganic cesium lead halide perovskite QDs.

5.2 Results

5.2.1 Self-assembling and characterizations of the perovskite QDs based particles (PQDsP)

We synthesized the all-inorganic perovskite QDs, CsPbBr$_3$, through quick injection of Cs-oleate solution (details can be seen in Chapter 2.2.3)\textsuperscript{124, 126}. The obtained perovskite QDs was dissolved into Hexane as shown in figure 5.1 (a). The Transmission Electron Microscopy (TEM) image of the synthesized perovskite QDs, CsPbBr$_3$, (figure 5.1 (d)) shows the size of the QDs around 10 nm which agrees with the reported results \textsuperscript{126, 262}. Next, we mixed the Hexane solution and mineral oil (with 0.2% Span 80) by 24 hours continuous-stirring allowing the Hexane to evaporate into the atmosphere (figure 5.1 (b)). As the evaporation process went on, the perovskite QDs based particles (PQDsP) were formed upon completion of the evaporation
Figure 5.1 Schematics and characterizations of the synthesized perovskite QDs based particles (PQDsP). (a) Synthesized CsPbBr$_3$ QDs in Hexane, (b) stir-mixing of CsPbBr$_3$ QDs in Hexane solution and mineral oil with 0.2% span 80, (c) Synthesized PQDsPs in mineral oil, (d) TEM image of the CsPbBr$_3$ QDs, (e) Size distribution PQDsP, (f) photo of the PQDsPs in mineral oil with 0.2% span 80, (g) PL spectra of the synthesized PQDsPs dispersed in mineral oil with 0.2% span 80, (h) & (i) are the images of PQDsPs under normal bright field microscopy and fluorescent microscopy configurations respectively. Scale bars in (h) (i) represent 20 µm.

as indicated in figure 5.1 (c). To illustrate the size of the synthesized PQDsP, we measured the size of the PQDsP under microscope and obtained the distribution by means of the Gauss fitting as shown in figure 5.1 (e). The result shows that the typical size of the particle synthesized through self-assembling is around 2.25 µm desirable for µPIV measurement of most of the circumstances. The bright emission of the synthesized PQDsP as illustrated in figure 5.1 (f) verifies its excellent fluorescent performance and hence potentials in µPIV related applications.
The photoluminescence (PL) of the PQDsP are shown in figure 5.1 (g). The luminescence demonstrates a strong green emission light with peak located at ~522 nm. The color of the emission light is most sensitive to most of the photodetectors and thus very suitable for imaging based applications. We observe PQDsPs under bright field microscopy (figure 5.1 (h)) and fluorescent microscopy (figure 5.1 (i)) configurations. The captured images exhibit a relatively uniform size distribution as well as bright and clear fluorescent particles, both of which endow PQDsPs desirable merits in µPIV

5.2.2 Micro-flow measurements in laminar flow systems

We firstly verify the measurement method via the PQDsP in a typical laminar flow system as shown in figure 5.2 (a). The fluid with PQDsPs inside flows through the microchip with a rectangular cross-section (Details can be seen in Chapter 5.4.1, & Video S 5.1). Due to the superior emission performance of the perovskite QDs, we obtained a clear and bright view of the PQDsPs inside the microchip through a home-built fluorescent optical microscopy setup (figure 5.2 (a)). Next, we process the fluorescent images via the particle correlation algorithm and calculate the flow field as plotted in figure 5.2 (b) & (c) (commercial software package, Dynamic studio, version 4.1). The vector field in figure 5.2 (b) offers the information on both direction and magnitude distribution of the velocity. The classic problem of single-layer-laminar flow at microscale follows the simplified Naiver-Stokes equation without the non-linear convection term due to the small Reynolds number (Re). The equation can be stated as \( \nabla p = \mu \nabla^2 \mathbf{u} \), where \( p \) is the hydrodynamic pressure, \( \mu \) and \( \mathbf{u} \) represent the dynamic viscosity and velocity field of the fluid. It interprets the physical process which essentially results from the balance between the hydrodynamic pressure driven and viscous dissipation. Theoretically, the flow velocity should follow a parabolic distribution over the microchip width (microchip geometries can be seen in Chapter 5.4.1) which matches the vector field in figure 5.2 (b). For the verification purpose, we carried out the computational fluid dynamics (CFD)
simulation and compare with our experimental result as demonstrated in figure 5.2 (c). The discrepancy between the experiments and the simulation may be caused by the selection of the measurement plane inside the microchip and is within acceptable range. We can conclude that the flow measurement enabled by the PQDsP offers a valid platform for quantitative flow measurement.

**Figure 5.2** PQDsP enabled quantitative measurement on laminar flow systems. (a) Fluorescent microscopy view of PQDsPs flowing in microchips, (b) vector field of the single-layer laminar flow, (c) flow profiles obtained from the measurement and CFD simulations Scale bars in (d) equal 50 µm.

Some crucial applications such as chemical reactions, drug delivery, cell processing, etc. generally require multi-step processing and the flexibility of varying conditions. Flow at microscale gives a niche for the aforementioned applications as a result of its small scale that can carry out the bio processing at the single-cell resolution\(^{246,261}\). All the advantages can be achieved by means of the interface control\(^{195}\). The hydrodynamic flow switching as the commonly adopted interface control technology, equips us with a simple and reliable method to manipulate the samples into various branches where distinct conditions can be designed for test or further processing. The hydrodynamic flow switching configuration consists of two cladding phase and one core phase (\(Q_{L1}, Q_{L2}\), and \(Q_c\) in figure 5.3 (a)). The detailed microchip geometry can be referred in Chapter 5.4.1, ‘Microchip fabrications’ part. We obtain the particle trace plots of the PQDsP by stacking the time frames. The results shown in figure 5.3 (a) clearly reveal a shifting of the core phase position from the centre line to the right end of the microchip (details can also be referred in Video S 5.2). Similar to the single-layer flow system, the
The hydrodynamic flow switching system is governed by the simplified Navier-Stokes equations:
\[ \nabla p = \mu \nabla^2 \mathbf{u}, \]
where \( p \) is the hydrodynamic pressure, \( \mu \) and \( \mathbf{u} \) represent the dynamic viscosity and velocity field of the two cladding phases (\( Q_{L1} \) and \( Q_{L2} \)) and one core phase (\( Q_C \)) with the PQDsPs. The system is balanced by the pressure \( p \), flow rates \( Q_i = \int u(x, y) \, dx \, dy \), where

\[ Q_1 = \int u_1(x, y) \, dx \, dy, \]
\[ Q_2 = \int u_2(x, y) \, dx \, dy, \]
\[ Q_C = \int u_C(x, y) \, dx \, dy. \]

**Figure 5.3** (a) Particle trace images of three-layer flow system under various flow ratios, (b) vector field of the three-layer flow system. The samples indicated in (a) are glycerol 65% in D.I. versus PQDsP in mineral oil with 0.2% span 80 versus glycerol 65% in D.I. Details can be seen in Chapter 5.4.2, Table 2. Scale bars in (b) equal 50 \( \mu \)m.

viscous dissipation and the interfacial conditions including \( \mathbf{u}_i = \mathbf{u}_{i+1} \) and

\[ \mu \frac{\partial U_1}{\partial y} = \mu_{i+1} \frac{\partial U_{i+1}}{\partial y} + \gamma \]

interprets the interfacial tension between the two neighbouring phases. We can easily adjust the position of the core phase by tuning the flow rates (\( Q_{L1} \), \( Q_{L2} \), and \( Q_C \)) and the fluidic properties (mainly the viscosity \( \mu_i \)). Hence, the investigation on the positional correlation with the flow rates and the quantitative flow field distribution becomes essential. Taking advantage of our synthesized PQDsP, we are able to capture both the interface
positions and the quantitative flow field distributions as shown in figure 5.3 (a) and figure 5.3 (b) (vector field for the condition of 100: 50: 100 µL/h)

Similar with the velocity distribution shown in figure 5.3 (b), we plot the velocity distributions under other flow rate ratios in figure 5.4 (a). We can observe a clear shift of the interface positions due to the tuning of the hydrodynamic pressure. The maximum velocities under other flow rate ratios are around 0.003 mm/s. Both the interfacial positions and the velocity profiles shows a validate measurement on the velocity distributions. We also conduct series of CFD simulations for comparison purposes (figure 5.4 (b)). The experimental results of the interfacial positions agree well with the simulations, which verify the feasibility of our method.

![Figure 5.4](image)

**Figure 5.4** (a) Velocity distributions of the laminar flow systems (figure 5.3 (a)) via PQDsP enabled quantitative measurement, (b) comparison of the interfacial positions between the experiments and simulations with a constant $Q_c=50$ µL/h.

### 5.2.3 Micro-flow measurement in droplets

Other than the abovementioned laminar flow systems, the droplet at microscale (also termed as droplet based microfluidics) has attracted enormous attentions as droplets are critical components in pharmaceuticals, medical diagnostics, food and cosmetic industries. The perfectly sized droplets generated in droplet microfluidics along with other associated manipulation methods enable the generation of new materials, provide novel avenues for chemical reactions, and achieve single-cell resolution of bioprocessing with the concept of
“Lab on a chip”. As the first step of the droplet based microfluidic, the monodispersed droplet formation presents itself an important position. Rising from all the geometrical study of the microchips, T-junction \(264, 265\) and flow focusing geometries \(266-268\) are widely adopted as a result of their simplicity and outstanding performances. Both geometries are capable of generating monodispersed droplet at a rate of a few thousand per second. The high generation rate benefits the aforementioned applications whereas it bring tremendous challenges to the fundamental investigations, as the high formation rate will inevitably introduce fast dynamics. So far quantitatively characterizing such fast dynamics remains difficult. Here we show that the synthesized PQDsP through self-assembling is promising in the field of high speed dynamic capturing. We demonstrate such capability of PQDsP in fast flow measurements for both geometries: T-junction and flow focusing.

In the droplet based microfluidic configuration, there are two phases: the carrier phase which stimulates the formation and the dispersed phase which breaks into individual droplets. We put the PQDsPs into the carrier phase for \(\mu\)PIV flow measurement in the T-junction geometry, as illustrated in figure 5.5 (a)-(f). We generate droplets by adopting different flow rate ratios of \(Q_d : Q_{ca} = 5:200 \ \mu\text{L/h} \) (figure 5.5 (a)), \(Q_d : Q_{ca} = 5:100 \ \mu\text{L/h} \) (figure 5.5 (b), (c)) and \(Q_d : Q_{ca} = 10:100 \ \mu\text{L/h} \) (figure 5.5 (d) ~ (f)) respectively. We can observe a clear increasing trend of droplet size with the increasing of the flow rate ratio. The droplet changes its shape from a circular shape (figure 5.5 (a)) to a long droplet plug train (figure 5.5 (d) ~ (f)). We are able to capture a clear and bright fluorescent image of the carrier phase with a black droplet inside at the exposure time of 100 \(\mu\text{s}\) which means we have achieved a quantitative measurement at the speed of 10,000 frames per second.
Figure 5.5 Droplet formations in T-junction geometry on different flow rates. (a), Droplet formation under the flow rate ratio of $Q_d:Q_c=5:200$ µL/h; (b), (c) Droplet formation under the flow rate ratio of $Q_d:Q_c=5:100$ µL/h; (d) ~ (e) Droplet formation under the flow rate ratio of $Q_d:Q_c=10:100$ µL/h. $Q_d$ and $Q_c$ illustrate the flow rate of the dispersed phase and the carrier phase respectively.

The flow filed condition of figure 5.5 (a) ($Q_d:Q_c=5:200$ µL/h) have been chosen for further quantitative analysis, as shown in figure 5.6 (a) ~ (d) and Video S 5.3. The flow field shown in figure 5.6 (b) is also obtained by cross correlation processing. We observe and measure the flow field near the interface of the droplet as enlarged by the view in figure 5.6 (c) & (d). The blockage of the droplet changes the flow distribution and forms the interesting patterns as demonstrated in figure 5.6 (c) & (d).
Figure 5.6 Ultra-fast flow measurement of droplets in microchips enabled by the PQDsP. (a) Fluorescent image of droplet generated in T-junction geometry, where PQDsPs are placed in carrier phase and the flow rate ratio is set to be $Q_c:Q_d=5:200$ µL/h, (b) flow field of the carrier phase, (c) and (d) enlarged view of the flow field at two critical positions.

For the other case, we intend to measure the flow field inside the droplet. Thus, we put the PQDsPs into the dispersed phase in the flow focusing geometry (figure 5.7 (a)). The detailed geometry and microchip design can be referred in Chapter 5.4.1, ‘Microchip fabrication’ part and Video S 5.4. Enabled by the PQDsP, we could observe the droplet clearly and quantitatively measure its corresponding flow field as presented in figure 5.7 (b). After a simple subtraction of the means velocity, the induced flow recirculation patterns are revealed to us (figure 5.7 (c)). A CFD simulation with the level set method is carried out for comparison purpose as plotted in figure 5.7 (d). We observe the flow recirculation patterns inside the
It can be seen that the flow patterns matches well with simulation. Details of the simulation are illustrated in Chapter 5.4.5.

Figure 5.7 (a) fluorescent image of the droplet in microchip with PQDsP in dispersed phase and the flow rate ratio being $Q_D:Q_{Ca} = 25:200~\mu\text{L/h}$, (b) flow field of the droplet indicated in (a), (c) velocity field after subtraction of the mean velocity to reveal the key flow patterns and the enlarged view of the flow recirculation patterns, (d) simulated result of the corresponding flow field in the droplet for comparison. $Q_D$ and $Q_{Ca}$ stand for the flow rate of the dispersed phase and the carrier phase respectively.
5.2.4 Applications of PQDsP in complex emulsion systems

The complex droplets, especially double emulsion (that is, a core droplet exists inside the outer droplet) are of increasing importance in drug delivery, encapsulations, food industries and dynamic optics \(^{222, 258, 259}\). In this article, we also conduct the µPIV measurement in the double emulsion by means of PQDsP. We design and implement the generation of the double emulsions with multiple and yet controllable numbers of core droplets inside. We manage to generate the double emulsion with 1~3 cores respectively (figure 5.8 (a), (c), (e), Video S 5.5). With the synthesized particle inside the outer droplet, we capture the fluorescent image and calculate out the flow field as plotted in figure 5.8 (b), (d) and (f) respectively. The influence brought by the occurrence of the core droplet interests us. Hence, we reveal the flow recirculation patterns by subtractions of the mean velocity. We could see flow recirculation patterns near each of the core droplets (figure 5.8 (g) ~ (i)). The PQDsP achieved ultra-high speed measurement will extend our capabilities in the fundamental studies which is of broad interesting to the field of microfluidics such as the interfacial phenomena related to the multi-phase systems (micro-droplets are a typical multi-phase system), transition from laminar to turbulent flow and the increasingly popular micro-encapsulation technologies. Furthermore, it will be a powerful tool to discover characteristics of flow in biological environments. For instances, the occurring organ on a chip concept\(^{269}\) helps to understand the functionality of specific organ and its working mechanism, among which the flow characteristics such as the blood velocity distributions and the incurred pressure distributions are of high interest to researchers. The proposed PQDsP offers great simplicity and high performance, which lead to vast potentials in practical applications as well.
Figure 5.8 Ultra-fast flow field measurement of the complex droplets (double emulsions). (a) Fluorescent image of the double emulsion with a single core, (b) the corresponding flow field of the droplet in (a); (c) & (d) are the fluorescent image and flow field of the double emulsion with double core respectively, (e) & (f) are the results of the double emulsion with tribble cores, (g) ~ (i) are the flow recirculation patterns within the tribble-core double emulsion revealed by subtraction of the mean velocity. The materials used for generation of the double emulsions are DI water with 2% (w.t.) Tween 20 as the inner or core droplet ($Q_{\text{core-droplet}}$), PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80 as the outer droplet ($Q_{\text{out-droplet}}$), 70% (w.t.) glycerol in DI water with 0.3% (w.t) SDS as the outer carrier phase ($Q_{\text{Ca}}$). The flow rate ratios for generation of the double emulsions are $Q_{\text{core-droplet}} : Q_{\text{out-droplet}} : Q_{\text{Ca}} = 10.5 : 22 : 350 \, \mu L/h$ for one core, $Q_{\text{core-droplet}} : Q_{\text{out-droplet}} : Q_{\text{Ca}} = 12 : 25 : 350 \, \mu L/h$ for two cores, $Q_{\text{core-droplet}} : Q_{\text{out-droplet}} : Q_{\text{Ca}} = 12 : 25 : 250 \, \mu L/h$ for three cores respectively.

5.3 Discussion and summary

The high speed quantitative measurement of the flow field holds significant impact to both the fundamental mechanism studies and the practical applications in the field of microfluidics. Fundamental studies at microscale, such as multi-phase laminar flow and the carrier-dispersed induced droplet systems, require in-depth understanding of the flow field distributions to gain knowledge such as the influences of the interfaces on the flow velocity distributions, characteristics of vortices formation and the their influences to the flow. Down to the microscale, the applications such as the chemical reaction, material synthesis, high efficient sorting, fulfilling of the “organ on chip” design and on chip cell sequencing rely heavily on the flow field. For instances, the fast and high efficient mixing are critical and yet challenging for
practical applications in chemical reaction and material synthesis at microscale as a result of small Re flow which lacks sufficient convection flow. Instead, researchers proposed vortex induced mixing or mixing prompted by external fields. Similarly, for the scenarios of on chip bio-engineering, the key processing would be the controlling of the cell under the microchip environment. As refereed in the literature, the cell was encapsulated by droplets which was later merged by external electric fields and sequenced accordingly. A high speed processing rate is commonly considered to enhance the productivity. However the high processing rate will level up the flow induced stress which determines the survivability of the cell during the encapsulation and merging process. Investigations of such process, hence the design and optimization of whole chip based bio-processing require a comprehensive understanding of the flow field. Among all the technologies, the fluorescent particle enabled µ-PIV is the very best approach so far because it is most powerful and widely adopted technology that offers quantitative flow vector distribution (including both the magnitude and direction). Our work also provides the insights for further improvement of the µ-PIV technologies, which are introducing advanced quantum materials with high PLQY and the synthesis procedure for the trace particle by means of self-assembling. Rising from the requirements of the imaging correlation algorithm and the resolution of the modern high speed cameras, the common time scale for measurement such as the fast dynamics within the droplet, high efficient sorting, cell encapsulation processing and merging triggered by the electric field, can reach up to tens of microseconds (in the order of 100,000 frames per second) depending on the hydrodynamic conditions. Failed to match the dynamic time scale will result in the missing of the flow field distribution not to mention the key information such as the recirculation patterns, the stress levels endured by the cell, etc. The commercially synthesized fluorescent particles suffer from the relatively low emission intensity due to surface coating method which leads to the insufficiency of the fluorescent materials. The incurred relatively low measurement speed
largely hinders the application scope of this technology. Currently most high speed μ-PIV systems choose high frequency, high power, multi-cavity, pulse lasers to enhance the capturing speed. Even though such laser sources will inevitably incur problems such as joule heating, emission instability and above all the high cost and complexity of the system, the typical measurement speed can only achieve a few thousand frames per second depending on the operation conditions. In this work, we try to tackle the challenge by another approach: improving the fluorescent tracer particles. We employed a novel material, all-inorganic CsPbBr$_3$ QDs, and took advantage of their high PLQY (around 90%). Although all-inorganic perovskite QDs are emerging as a potential materials in fields of solar cell, LED, and lasers, we are the first to utilize them in the field of microfluidics. Furthermore, instead of using the conventional surface coating method, we developed a new self-assembly method to fabricate the tracer particles from CsPbBr$_3$ colloidal QDs. We are able to assemble CsPbBr$_3$ colloidal nanocrystals into micron sized particles without fluorescence quenching, desirable for μ-PIV measurement on fast flow in microfluidics. Compared to the conventional procedure of fabricating tracer particles that needs an extra coating, our strategy is much more cost effective, and more importantly provides higher fluorescence intensity which is decisive to high speed measurements.

In the laminar flow system (figure 5.2 & 5.3), we measured the flow field of the layer with the PQDsPs and record the interface positions. The comparison with CFD simulations (figure 5.4) proves the validity of our method. While in the droplet based microfluidic scenarios (figure 5.5 ~ figure 5.7), we are able to measure the flow fields for cases of carrier phase, and dispersed phase in one single droplet and complex droplets (double emulsions), respectively. Endorsed by the high PLQY of perovskite QDs, CsPbBr$_3$, and the novel approach for particle synthesis, self-assembling, we achieved an unprecedented ultrafast quantitative measurement speed at 10,000 frames per second with a single CW laser with power of only 67 mW which is much
lower than the commercial high speed laser system whose power is normally above the order of 10 W. Certainly with our approach, the speed can be further increased by employing a laser with higher power.

5.4 Materials, methods and simulations

5.4.1 Microchip fabrications

The microchips were fabricated by lithographic method which utilized high precision chrome masks to transfer the designed patterns onto the silicone based wafers. Followed by the polydimethylsiloxiane (PDMS, Dow Corning Sylgard 184) curing process and oxygen plasma bonding, the patterns were engraved into the microchips. We utilized the UV-initialized polymerization method for surface modification to facilitate the droplet formation of organic phase in PDMS based microchips (see Chapter 2.6 for details). The designed geometries employed in our experiments can be seen in figure. 5.9. We designed and fabricated 5 types of microchips with distinct function as following: figure 5.9 (a) and (b) are designed for single and three-layer flow experiments respectively, (c) and (d) are built for the measurement of droplets in T-junction and flow-focusing configuration respectively, and (e) is fabricated for the generation of complex droplets, specifically double emulsions.
Figure 5.9 Schematics of the microchip designs adopted in the experiments. (a) Microchip utilized in single layer laminar flow, (b) microchip design for the three-layer flow experiments, (c) geometries for the droplet generation in T-junction, (d) microchip design for droplet formation in flow focusing configuration, (e) microchip employed for formation of complex droplets (double emulsions).
### 5.4.2 Fluidic sample preparation for the experiments

**Table 2** Fluidic samples list for the experiments

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of the exp.</th>
<th>Fluidic samples</th>
<th>Schematics of the design</th>
<th>Results in Main Article</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single layer laminar flow</td>
<td>PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80</td>
<td>Figure. 5.9 (a)</td>
<td>Figure 5.2</td>
</tr>
<tr>
<td>2</td>
<td>Three layer laminar flow</td>
<td>Core phase: PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80 Cladding phase: 65% (w.t.) glycerol in deionized (DI) water</td>
<td>Figure. 5.9 (b)</td>
<td>Figure 5.3</td>
</tr>
<tr>
<td>3</td>
<td>Droplet formation in T-junction</td>
<td>Dispersed phase: 65% (w.t.) glycerol in DI water Carrier phase: PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80</td>
<td>Figure. 5.9 (c)</td>
<td>Figure 5.5 &amp; 5.6</td>
</tr>
<tr>
<td>4</td>
<td>Droplet formation in flow-focusing</td>
<td>Dispersed phase: PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80 Carrier phase: 70% (w.t.) glycerol in DI water with 0.3% (w.t) sodium dodecyl sulfate (SDS)</td>
<td>Figure. 5.9 (d)</td>
<td>Figure 5.7</td>
</tr>
<tr>
<td>5</td>
<td>Complex droplet (Double emulsions)</td>
<td>Inner droplet: DI water with 2% (w.t.) Tween 20 Outer droplet: PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80 Outer Carrier: 70% (w.t.) glycerol in DI water with 0.3% (w.t) SDS</td>
<td>Figure. 5.9 (e)</td>
<td>Figure 5.8</td>
</tr>
</tbody>
</table>

**Note:**

1. Details of the PQDsPs solution processing can be referred in “Materials and Methods” in Chapter 5.2.1;
2. Mineral oil (M5904), glycerol, Span 80, SDS and tween 20 are all purchased from Sigma Aldrich;
3. DI water is obtained from Merck MilliQ system Grade I.
5.4.3 Interfacial tension characterizations

Table 3 Interfacial tension characterizations for the fluidic systems adopted in the experiments

<table>
<thead>
<tr>
<th>No.</th>
<th>Two fluidic samples that form the interface</th>
<th>Interfacial tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80, DI water with 2% (w.t.) Tween 20</td>
<td>13.985±1.245</td>
</tr>
<tr>
<td>2</td>
<td>PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80, 70% (w.t.) glycerol in DI water with 0.3% (w.t) SDS</td>
<td>0.754±0.008</td>
</tr>
<tr>
<td>3</td>
<td>PQDsPs in Mineral Oil with 0.2% (w.t.) Span 80, 65% (w.t.) glycerol in DI water</td>
<td>9.806±0.201</td>
</tr>
</tbody>
</table>

Note: The interfacial tensions are measured by the pendent droplet method using a tensiometer (model: FTA200). We measured the interfacial tension for at least three times to ensure the repeatability.

5.4.4 Microfluidic configurations

The fluidic samples were loaded into high precision glass syringes (Hamilton Gastight 1001), whose flow rates were controlled by high precision, pulse free syringe pumps (Centoni neMESYS). The samples were then flowing into the microchip via chemical protective PTFE microtubings (Cole-Parmer). The final results of the PQDsP flowing through the microchip were illuminated by the emission of the synthesized particles which were excited by a CW 442 nm laser line with the power of 67 mW, imaged via a home-made fluorescent microscopy setup, and recorded by a high speed camera (phantom m311).

5.4.5 Computational fluid dynamics (CFD) simulations---the model of velocity field and interface tracking

In this model, the flow field of the multiphase fluids is calculated with two sets of governing equations within each fluid. The continuity and momentum equations can be defined as:

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{u}) = 0
\]

\[5.1\]
\[
\frac{\partial}{\partial t}(\rho \ddot{u}) + \nabla \cdot (\rho \ddot{u}) = -\nabla P + \nabla \cdot \left[ \mu \left( \nabla \ddot{u} + \nabla \ddot{u}^T \right) \right] + \mathbf{F} \tag{5.2}
\]

where \( \rho \) is fluid density, \( t \) is the time, \( \ddot{u} \) is the velocity vector, \( P \) is the pressure and \( \mathbf{F} \) is the source term represents the external volume force exerting on the liquid.

In our simulation with negligible gravitational force, the source term is mainly the interfacial force \( \vec{F}_\sigma \) which can be determined using the surface tension force (CSF) model:

\[
\vec{F}_\sigma = -\sigma \kappa \hat{N}_F D\left(\vec{x} - \vec{x}_f\right) \tag{5.3}
\]

\[
\hat{N}_F = \frac{\nabla \phi}{|\nabla \phi|} \tag{5.4}
\]

\[
\kappa = \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|} \tag{5.5}
\]

\[
D\left(\vec{x} - \vec{x}_f\right) = \begin{cases} 
(1 + \cos(\pi \phi / \varepsilon)) / (2\varepsilon), & \text{if } |\phi| < \varepsilon \\
0, & \text{otherwise}
\end{cases} \tag{5.6}
\]

For the multiphase flow system, the properties of the liquid jump across the interface. The discontinuity of the physical properties can be treated by using the smoothed Heaviside function \( H(\phi) \) by either an arithmetic mean Eq.5.7 or a harmonic mean Eq.5.8.

\[
\alpha(\phi) = H\alpha_+ + (1 - H)\alpha_- \tag{5.7}
\]

\[
\frac{1}{\alpha(\phi)} = \frac{H}{\alpha_+} + \frac{(1 - H)}{\alpha_-} \tag{5.8}
\]

Normally, the arithmetic mean is used to calculate for the value of density. The harmonic mean appears more accurate for the calculation of viscosity. The smoothed Heaviside function \( H(\phi) \) is expressed as \( H(\phi) = \begin{cases} 
0, & \text{if } \phi < -\varepsilon \\
(\phi + \varepsilon) / (2\varepsilon) + \sin(\pi \phi / \varepsilon) / (2\pi), & \text{if } |\phi| < \varepsilon \\
1, & \text{if } \phi > \varepsilon
\end{cases} \tag{5.9} \)
The interface between the oil and the mixture of water and glycerol is tracked by the level-set method (LSM) with the variable \( \phi(x, t) \) defining the interface. The level-set function \( \phi(x, t) \) is a signed normal distance function from the interface:

\[
\phi(x, t) = \begin{cases} 
< 0, & \text{if } x \in \Omega, \\
0, & \text{if } x \in \Gamma, \\
> 0, & \text{if } x \in \Omega, 
\end{cases} \tag{5.10}
\]

The positive value represents the phase inside the interface, while the negative value states for the phase outside the interface. The interface exhibits function value at 0. The motion of the interface is obtained by taking the time derivative of \( \phi(x, t) = 0 \),

\[
\frac{\partial \phi}{\partial t} + \vec{u} \cdot \nabla \phi = 0 \tag{5.11}
\]

This function is normally solved locally around a narrow band across the interface for saving the computational time without affecting the accuracy.

During the calculation procedure, a reinitialization procedure is applied to reset the level-set function to make sure it to be a signed distance function of the interface and satisfy \( |\nabla \phi| = 1 \).

The following reinitialization equation is used for the correction of \( \phi \) at time \( t^* \):

\[
\frac{\partial \phi^*}{\partial t^*} = \text{sign}(\phi_c)(1 - |\nabla \phi^*|) \tag{5.12}
\]

\[
\phi_c(x, t) = \phi(x, 0) \tag{5.13}
\]

\[
\text{sign}(\phi_c) = \phi / \sqrt{\phi^2 + |\nabla \phi|^2 \Delta x} \tag{5.14}
\]

The governing equations the fluid flow Eq. 5.1 and 5.2 are solved on the Cartesian staggered grid by finite volume method (FVM). The computational domain is uniformly divided into a number of control volumes as shown in figure 5.10. The corresponding velocity profile in the
channel is presented in figure 5.11 with flow rate ratio at 175:50:25 μL/h. We utilize the SIMPLER algorithm for coupling of velocity and pressure and second-order accurate total variation diminishing discretisation schemes for convection-diffusion effect. We adopt the particle level-set method to ensure the mass conservation. The interface distribution at different flow rate ratio is plotted in figure 5.12.

**Figure 5.10** Uniform mesh used in the calculation.

**Figure 5.11** The velocity distributions.
Figure 5.12 Interface distributions at different flow rate ratios.
Chapter 6. Summary and Prospect

6.1 Summary

We have employed two kinds of fluorescent material e.g. carbon dots and self-assembled CsPbBr3 Perovskite seeding-particles to visualize the microfluidics. The unclear emission mechanism of carbon dots greatly hinder the applications of carbon dots. Thus we first explore the emission mechanism of carbon dots as depicted in Chapter 3. The interface capture/visualization and the quantitatively velocity measurements have been demonstrated successfully by utilizing carbon dots and carbon dots based seeding particle. The results in Chapter 4 imply the capability of carbon dots based microfluidic visualization, especially for the bio-microfluidics. To address the high speed quantitatively velocity field measurement issue in microfluidics, we synthesize the CsPbBr3 Perovskite microparticle, as presented in Chapter 5.

6.1.1 Carbon dots emission originate from self-trapped exciton

Carbon dots employed in the experiments are synthesized by microwave heating method. The polarization spectroscopy and electric field modulation spectroscopy indicate that carbon dots emission is a kind of localized emission, power dependent PL measurement further confirm that it is the self-trapped exciton which contribute carbon dots emission, based on the self-trapped exciton model, both the time-resolved measurement, excitation dependent PL, and decreased linear polarization value for increased excitation wavelength can be interpreted consistently. The electronic structure of carbon dots is illustrated in figure 3.13, and enlighten by the self-trapped exciton model in SiO$_2$ [179, 184], we suggested the possible self-trapped exciton structure in carbon dots that is the ruptured C-O bond and/or the peroxy radical bond(one O atom inert into the C-O bond), as sketched in figure 3.14.
6.1.2 Dynamic Visualizations in Microfluidics Enabled by Fluorescent Carbon Nanodots

The exploration of carbon dots emission mechanism in Chapter 3 triggered the microfluidic applications in Chapter 4. We evaluated the photo-stability, chemical inertness, and biocompatibility previous to the microfluidic visualization. Mixing dynamics and cladding interfacial positions control have been demonstrated by using carbon dots as the fluorescent marker. In addition, the droplet dynamic including monodispersed droplet generation, double component droplet generation, merging of the double component droplet and double emulsion droplet have been investigated by utilizing carbon dots. The carbon dots based fluorescent seeding particle allows the quantitatively measurement in microfluidics.

6.1.3 Quantitative Investigation of Fast and Complex Flow Field in Microfluidics: Self-assembled CsPbBr3 Perovskite Flow Tracers

To match fast dynamics in microfluidics, high-speed µPIV with fluorescent seeding particles is highly demanded. We take advantage of the high photoluminescence quantum yield of inorganic CsPbBr3 quantum dots as well as the novel assembly method to enable µPIV with enhanced performance. As such, we have achieved an unprecedented ultrafast measurement speed of 10,000 FPS, which represents a remarkable improvement in performance of µPIV and allows us to explore some fast and complex phenomena in microfluidics. More importantly, this measurement speed was achieved by employing a single CW laser with power of only 67 mW which is much lower than a laser used in a commercially available high speed µPIV system.

We have demonstrated the parallel laminar flows and droplet (for both oil and water droplet) velocity measurement by adapting the self-assembled seeding particles, and the simulation results match very well with the experimental observations. The complex double emulsion system measurement results are also illustrated in figure 5.8. All of these experiment and simulation results unambiguously suggested the capability and effectiveness of our proposed tracer particles.
6.2 Outlooks

We have explored different types of fluorescent tracers, thus the next step is to apply these fluorescent materials into the distinct practical applications. It is well known that microfluidic platform have tremendous application in biology such as cell subpopulations, Single-Cell Transcriptomics\textsuperscript{271}, single-cell analysis,\textsuperscript{74} Genome-wide Expression Profiling\textsuperscript{272}, Clinical-scale generation of platelets\textsuperscript{273} and etc. While, as we discussed previously, the organisms perform different behaviors at micro scale compared to macro scale, so it's reasonable to speculate that the performance of the organisms e.g. cells, bacteria etc. are velocity field dependent. Consequently, we want to explore how the velocity filed and microenvironment can influence the bio assay results, where the velocity filed can be controlled by the hydrodynamic pump and measured by the fluorescent seeding particles, and the various microenvironment can be constructed by the proper micro chip design.

In chapter 5, we have achieved the self-assembled oil phase based fluorescence seeding particles. Oil phase is one of the most important phases in microfluidics, while in addition to the oil phase, aqueous phase is also another key phase. However, for the water phase based flow field quantitative measurement, the seeding particles are still synthesized by coating fluorescent materials (dyes or semiconductor quantum dots) onto the surface of non-fluorescent spheres. The non-fluorescent sphere based seeding particles can meet the requirement of applications in the fields of relative low speed region, but it’s not capable of high speed flow field quantitative measurement. Consequently, similarly to the self-assembled inorganic CsPbBr\textsubscript{3} micro tracer, we want to explore the self-assembled fluorescent micro seeding particles but in aqueous phase, then we can explore the high speed fluid dynamics and applications at micro scale for the whole fluid flow (both oil phase and aqueous phase).

Due to the striking advantages of micro-fluidics, numerous applications based on this platform are explored, what’s more excitation is that some of the microfluidic applications are
commercialized. While as pointed out by George Whitesides and other researchers, we need continue to find some killer applications or prove solutions which outperform current utilized methods, after that, the all biologists, chemists and companies will go small scale, as said by Nathan Blow.

References

50. X. C. i Solvas, *Chemical Communications,* 2011, **47**, 1936-1942.