

Polish Academy of Sciences
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Droplet Generation in Microfluidic Cross-Junctions: Mechanisms and Applications as Cell Incubators

*Wytwarzanie kropeł w mikroprzepływowych złączach krzyżowych: mechanizmy i
zastosowania jako inkubatory komórkowe*

Submitted for
PhD Thesis

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Warsaw, 2024

Acknowledgements

First and foremost, I want to extend my deepest gratitude and warmest thanks to my supervisor, Piotr M. Korczyk, for granting me the opportunity to pursue a Ph.D. in Poland and for his unwavering guidance. I am profoundly grateful for his invaluable mentorship and encouragement throughout my studies. His insights and feedback have been instrumental in shaping this work. My heartfelt thanks also go to my auxiliary supervisor, Sławomir Bloński, whose expertise in experimental and fabrication techniques has significantly enriched my skills and knowledge. I deeply appreciate the mentorship I have received from both of you.

I would like to thank all the members of the Department of Biosystems and Soft Matter, where I was fortunate to work alongside incredible colleagues. A special thanks to Prof. Tomasz Lipniacki and Prof. Maria Ekiel-Jezewska for their invaluable discussions and encouragement during my studies.

Beyond my department, I am grateful for the support of Dariusz Jarzabek's group and Magdalena Osial's group. I also extend my thanks to all my fellow Ph.D. students at IPPT-PAN, and I wish everyone a bright and successful future, no matter the challenges ahead.

Outside the institute, I would like to express my heartfelt gratitude to my wonderful friends, Robert Okabe-Antosik and his family and Mikołaj Dakowski and his family, for their kindness, friendship, and support, which have brightened my life beyond the lab. I also want to thank Daniel Martinez Sierra and Hengky Leo, whose friendship and encouragement have meant so much to me.

Special thanks go to my wife, Elisa, and my children, Ganapatih and Kinara, whose unwavering support, patience, and love have been my greatest sources of strength throughout this journey. I am forever grateful to my mother, who has always provided me with constant encouragement and prayers despite being far away and not in her best health. To all my family members, thank you for your enduring love and support, which have kept me grounded and motivated through the highs and lows of my Ph.D. journey.

Finally, I would like to express my deepest gratitude to God for blessing me with the opportunity to conduct research, earn my Ph.D. degree, and experience life in Poland with my family.

Abstract

Droplet microfluidics is an emerging technique that has revolutionized biomedical research by enabling precise high-throughput techniques for single-cell analysis, drug delivery, and point-of-care diagnostics. This technique relies on the production of small, isolated compartments formed by immiscible fluids, typically oil and water, within a network of microscale channels on a chip. Most droplet microfluidics platforms utilizing passive-type droplet generators operate in a limited operating range, the so-called squeezing droplet formation, where the formation process is dominated by interfacial forces over viscous forces. It is understood that the squeezing droplet formation is insensitive to the relative magnitude of these forces, captured by the capillary number (Ca), making the squeezing droplet formation a favorable operating range as it is less sensitive to the fluid properties. However, the droplet formation at a lower range of capillary numbers shows a significant deviation from the squeezing regime behavior, which is not well understood. In this study, droplet formation in microfluidic cross-junctions at very low Ca is investigated. An initial investigation found that another droplet formation mode exists below the operating range of squeezing droplet formation, where the volume of the produced droplets strongly depends on Ca . This flow pattern is known as the leaking regime, which was recently discovered for droplet production in T-junctions. This study reveals the fundamental difference in leaking droplet formation between cross-junctions and T-junctions. In cross-junctions, the neck of the forming droplet may elongate substantially before it breaks, and in contrast to the case of the T-junction, the magnitude of this elongation depends strongly on the value of the capillary number. This elongation significantly affected the final droplet volume in the low-capillary-number regime. To understand this process, a mathematical analysis was conducted to clarify the dependency of droplet elongation on the capillary number. A generalized scaling law was proposed for the droplet volume in microfluidic cross-junctions and validated using experimental data obtained from cross-junction devices with various cross-sectional geometries. The results advance the understanding of droplet formation mechanics in a very low capillary number range. Additionally, this study also discusses the practical aspects of using droplet microfluidics as a cell culture micro-sized incubator. Effective methods for minimizing the loading time of the cell-containing medium and droplet liquid mass transport through the permeable PDMS material are suggested. The resulting microfluidic platform was shown to sustain the lives of most cells for more than a day.

Streszczenie

Mikroprzepływy kropelkowe to stosunkowo nowa i dynamicznie rozwijająca się technologia, która zrewolucjonizowała badania biomedyczne. Umożliwia ona precyzyjne, wysokoprzepustowe analizy pojedynczych komórek, dostarczanie leków oraz diagnostykę w punktach opieki. Technika ta opiera się na wytwarzaniu małych, odizolowanych objętości cieczy powstających z niemieszających się płynów, zazwyczaj oleju i wody, w sieciach mikroskalowych kanałów utworzonych w układach mikroprzepływowych. Większość mikroprzepływowych platform kropelkowych z pasywnymi generatorami kropelek działa w ograniczonym zakresie roboczym, w tzw. reżimie „squeezing”. W procesie formowania kropelek w tym reżimie oddziaływania międzyfazowe dominują nad oddziaływaniami lepkościowymi. Przyjmuje się, że formowanie kropli w reżimie „squeezing” jest niewrażliwe na względną wielkość tych sił, opisaną liczbą kapilarną (Ca). Dzięki temu reżim ten jest korzystny, ponieważ formowanie kropli jest mało wrażliwe na właściwości cieczy, a ich wielkość można regulować, zmieniając stosunek obu przepływów. Jednakże tworzenie kropli w zakresie niższych liczb kapilarnych wykazuje znaczne odchylenie od zachowania typowego dla reżimu „squeezing”, co nadal nie jest dobrze zrozumiane. W niniejszym badaniu zbadano proces tworzenia kropli w mikroprzepływowych połączeniach krzyżowych przy bardzo niskich wartościach Ca . Wstępne wyniki wykazały, że poniżej typowego zakresu reżimu „squeezing” występuje inny tryb formowania kropli, w którym ich objętość silnie zależy od liczby kapilarnej. Wzorzec ten, znany jako „leaking”, został niedawno opisany w kontekście tworzenia kropli w złączach T. Niniejsze badanie ujawnia fundamentalną różnicę w mechanizmie tworzenia kropli między połączeniami krzyżowymi a złączami T. W połączeniach krzyżowych szyjka formującej się kropli może znacznie się wydłużyć przed jej oderwaniem. W przeciwieństwie do złączy T, wielkość tego wydłużenia zależy istotnie od wartości liczby kapilarnej. Wydłużenie to znacząco wpływa na końcową objętość kropli w reżimie niskich wartości Ca . Aby zrozumieć ten proces, przeprowadzono analizę matematyczną, która wyjaśnia zależność wydłużenia kropli od liczby kapilarnej. Zaproponowano uogólnione prawo skalowania dla objętości kropli w mikroprzepływowych połączeniach krzyżowych, a następnie zweryfikowano je na podstawie danych eksperymentalnych uzyskanych z urządzeń o różnych geometriach przekroju. Uzyskane wyniki przyczyniają się do lepszego zrozumienia mechaniki formowania kropli w bardzo niskim zakresie liczby kapilarnej. Dodatkowo, badanie porusza praktyczne aspekty wykorzystania mikroprzepływów kropelkowych jako inkubatorów komórkowych. Zaproponowano skuteczne metody minimalizacji czasu ładowania pożywki zawierającej komórki oraz transportu masy cieczy w postaci kropli przez przepuszczalny materiał PDMS. Wykazano, że opracowana platforma mikroprzepływowa może utrzymać żywotność większości komórek przez ponad 24 godziny.

List of Publications

The following peer-reviewed publication present the main results and methods discussed in this work:

- **T. Kurniawan**, M. Sahebdivani, D. Zaremba, S. Blonski, P. Garstecki, V. van Steijn, P.M. Korczyk, Formation of droplets in microfluidic cross-junctions at small capillary numbers: Breakdown of the classical squeezing regime, *Chem. Eng. J.* 474 (2023) 145601. <https://doi.org/10.1016/j.cej.2023.145601>.

Conference posters or presentations related to this work:

1. **T. Kurniawan**, P. Koza, M. Milczarek, P. M. Korczyk, T. Lipniacki, Technical notes towards an efficient experiment using droplets as incubators for the immunology study, Warsaw Soft Matter day, 2023-10-04, Warsaw (PL).
2. **T. Kurniawan** and P. Korczyk, A scaling law for the neck-shape in microfluidic flow-focusing device, ICTAM2021, 25th International Congress of Theoretical and Applied Mechanics, 2021-08-22/08-27, Milano (virtual) (IT), pp.1167-1168, 2021
3. **T. Kurniawan** T and P. M. Korczyk, Formation of dual-size droplets in the microfluidic cross-junction device, NanoBioTech-Montreux, The 24th Annual European Conference on Micro and Nanoscale Technologies, 2021-11-15/11-17, Montreux (CH), pp.1-2, 2021
4. **T. Kurniawan** and P. Korczyk, Transitional regime of droplet generation in a microfluidic flow-focusing device. 6th International Conference on Nano and Micromechanics, Rzeszów, Poland (3 – 5 July 2019).
5. **T. Kurniawan** and P. Korczyk, The effect of aspect ratio to droplet generation in cross-junction microfluidic device. Summer School: Quality in research and development - standards, norms and quality certifications, Warsaw, Poland (12 – 14 June 2019).

The following are peer-reviewed papers published during the PhD study

1. R. Dumbill, J. Rabcuka, J. Fallon, S. Knight, J. Hunter, D. Voyce, J. Barrett, M. Ellen, A. Weissenbacher, **T. Kurniawan**, S. Blonski, P. M. Korczyk, R. Ploeg, C. Coussios, P. Friend, P. Swietach, Impaired O₂ unloading from stored blood results in diffusion-limited O₂ release at tissues: evidence from human kidneys. *Blood*, 2024, 143(8), pp. 721–733. <https://doi.org/10.1182/blood.2023022385>.
2. **T. Kurniawan**, P.-H. Tsai, S.-S. Chen, D.H. Frakes, C.-C. Chen, A.-B. Wang, Practical notes toward higher quality and more reliable experiments on drop and liquid surface interactions, *Exp. Fluids*. 63 (2021) 8. <https://doi.org/10.1007/s00348-021-03346-w>.
3. T. Suwa, **T. Kurniawan**, Redesigning a commercial combined cycle in an undergraduate thermodynamics course: Connecting theory to practical cycle design, *Int. J. Mech. Eng. Educ.* 49 (2021) 448–467. <https://doi.org/10.1177/0306419020904647>.

4. 1. L. Anggraini, A.N. Wahyuni, R. Hernawan, **T. Kurniawan**, Performance Optimization of Power Plant Waste Heat Using H₂O-LiBr Absorption Refrigerant System, Indones. J. Comput. Eng. Des. IJoCED. 4 (2022) 30–37. <https://doi.org/10.35806/ijoced.v4i1.243>.

Fundings

1. Interaction of natural killer cells with infected cancer cell population: microfluidics-based experiments and single-cell-level mathematical modeling, National Science Centre (NCN), Grieg (Norwegian Financial Mechanism), 2020–2023. Location: IPPT-PAN, Warsaw, Poland.
2. Digital operations on droplets embedded into smart microfluidic architectures for applications in medical diagnostics and biological research. Foundation for Polish Science (FNP): First Team, 2018–2020. Location: IPPT-PAN, Warsaw, Poland.
3. Development and use of bioengineering and information technology methods in the prevention, diagnosis and therapy of civilization diseases - comprehensive development of professional competence of young scientific personnel Och! DOK. 2014–2020. (Project Och!DOK) Location: IBIB-PAN and IPPT-PAN, Warsaw, Poland.

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Nomenclature

A	Time-dependent factors scaling the shape of the neck in the direction perpendicular to the main channel axis [m]
$a = A/W$	Dimensionless A
a_0	Dimensionless A , at the initial time of the neck relaxation
A_G	The cross-sectional area of the gutter [m^2]
$b = \kappa/Ca$	Dimensionless fitting parameter that represents κ/Ca
$Ca = \frac{U_C \mu_C}{\gamma} = \frac{Q_C \mu_C}{WH\gamma}$	Capillary number
$D(t)$	Diameter of droplet
$D_0 = D(t = 0 \text{ s})$	initial diameter of droplet
E	Local maxima or minima of the width of the forming droplet [m]
H	The height of the microchannel [m]
$h(x)$	Approximation of the two-dimensional shape of the neck as it appears in the top-view image [m]
K_F	The curvature at the front of the forming droplet [1/m]
K_N	The curvature of the interface at the neck region [1/m]
L_0	The initial length of the gutter [m]
$l_0 = V_0/HW^2$	The dimensionless volume of the forming droplet at the start of the necking stage, $\tau = 0 \text{ s}$
$l_D = V_D/HW^2$	The dimensionless volume of the droplet
L_T	The distance measured from the tip of the growing droplet to the edge of the cross-junction inlet [m]
L_{T0}	The distance measured from the tip of the growing droplet to the edge of the cross-junction inlet at the beginning of the necking stage [m]
p	Constant parameter represents τ^* at the high Ca regime
$q = Q_D/Q_C$	Flow rate ratio between DP and CP
Q_B	Rate of flow of the CP bypassing a droplet through the gutters in the corners of the channel [m^3/s]
Q_C	Rate of flow of the CP [m^3/s]
Q_D	Rate of flow of the DP [m^3/s]
$Q_G = Q_B/4$	Rate of flow of the CP bypassing a droplet through the single gutter, [m^3/s]
Q_N	Rate of flow of the CP filling the volume V_N [m^3/s]
$\langle Q_N \rangle$	Time-averaged of the CP flow rate filling the volume V_N [m^3/s]
$q_N = Q_N/Q_C$	Fraction of CP flow that forms the neck
$\langle q_N \rangle$	Time-average of q_N
Q_O	The flow rate of the liquid exiting the chip, which is controlled in the negative pressure driving system

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r	radius of Si-wafer [m]
S_{ang}	angular velocity of the spin coater [RPM or rad/s]
t	Time [s]
$t^* = tQ_C/W^2H$	Dimensionless time
$U_C = Q_C/WH$	The mean velocity of the CP [m/s]
V_0	The volume of a forming droplet at the end of the filling stage [m ³]
$v_0 = a_0\omega_0$	Dimensionless volume at the initial time of neck relaxation
V_D	The final volume of a droplet [m ³]
V_N	The volume of the CP collected behind the forming droplet [m ³]
$v_N = V_N/HW^2$	The dimensionless instantaneous volume of the CP collected behind the forming droplet
$V_{N0} = V_N(\tau)$	The volume of the CP collected behind the forming droplet at the end of the necking stage [m ³]
$v_{N0} = V_{N0}/HW^2$	The dimensionless volume of the CP collected behind the forming droplet at the end of the necking stage
W	Width of the channel [m]

Greek Symbols

α	The correction factor of the specific cross-section of the gutter
β	A constant parameter in the T-junction device that approximates the boundary between the squeezing and leaking regime
γ	Interfacial tension [N/m]
$\Delta p_L = \gamma(K_F - K_N)$	Pressure drop over a droplet due to the Laplace pressure [N/m ²]
Δp_G	Pressure drop due to viscous flow through a gutter [N/m ²]
δ	Dimensionless width of the droplet filament
ε	Dimensionless length of the droplet filament
Θ	The area under the neck measured from an image [m ²]
$\theta = a\omega \approx \frac{\Theta}{HW^2}$	The dimensionless area under the neck shape
κ	The dimensionless coefficient represents the average changes in necking flow rate due to the variation of the gutter geometry and droplet curvature
κ_0	The dimensionless coefficient describing the relation between the initial droplet/gutter length and the capillary number
Λ	The coefficients of the length dimension characterizing the geometry-dependent shear magnitude in the direction perpendicular to the main channel axis [m]
$\lambda = \Lambda/W$	Dimensionless Λ
λ_0	A constant parameter represents the value of the initial droplet length, l_0 , at the higher Ca regime
μ_C	The dynamic viscosity of the continuous phase [Pa s].
μ_D	The dynamic viscosity of the droplet phase [Pa s].
Ξ	The coefficients of the length dimension characterizing the geometry-dependent shear magnitude in the direction along the main channel axis [m]
$\xi = \Xi/W$	Dimensionless Ξ

ρ	The dimensionless volume of the droplet filament
ρ_0	The initial dimensionless volume of the droplet filament
π	Mathematical constant ≈ 3.14
τ	Duration of the necking stage [s]
$\tau^* = \tau \frac{Q_C}{W^2 H}$	Dimensionless duration of the necking stage
τ_{HL}^*	Dimensionless time when q_N reaches 0.5
$\tilde{\tau} = t\gamma/\mu_C W$	Dimensionless time related to the relaxation experiment
χ	A dimensionless coefficient represents the strength of neck-shape elongation
ψ	The slope of a versus ω , $da/d\omega$
Ω	Time-dependent factors scaling the shape of the neck in the direction along the main channel axis [m]
$\omega = \Omega/W$	Dimensionless Ω
ω_0	Dimensionless Ω at the initial necking or relaxation process
$\langle . \rangle = \frac{1}{\tau} \int_{t=0}^{t=\tau} . dt$	Time-average of quantity $\{.\}$ over the necking stage duration

Subscript and superscript

0	A quantity at the beginning of the necking stage $t = 0$, or at any beginning of the process described in the text
*	Denotes dimensionless time
B	Bypass
C	Continuous phase
D	Droplet phase
G	Gutter
HL	A quantity at the time when q_N reaches 0.5
L	Laplace pressure
N	Necking
N0	A quantity at the end of necking stage $t = \tau$
T	Tip of the droplet
T0	Tip of the droplet at the beginning of the necking stage $t = 0$

Abbreviations

CP	Continuous phase
DP	Droplet phase

Chapter 1

Introduction

1.1 A Brief History of Microfluidics

Microfluidics is a science and engineering of fluid manipulation in micro or nano-sized channels. It is a small – sized system consisting of channel(s) and component(s) that performs certain kind of protocol, for example: mixing of fluids, creating droplets, manipulating micrometer-sized material such as cells, DNA, etc. A microfluidic network resembles an electronic chip, but with fluids flowing within it instead of an electric current. A schematic showing the progress of the microfluidic field from its infancy to the present is shown in Fig. 1.1.

The period between 1980 and 1990 saw significant foundational developments of the microfluidic field. The field itself has its origin back to the late 70s, i.e., from the success stories of microfabrication technique in the development of miniaturized gas chromatography [1] and ink-jet printers [2]. The initial follow-up includes the development of various silicon-based micropumps [3], microvalves and other essential components for fluid control, termed initially as “Micro Liquid Handling Devices” [4] and later as “Microfluidic” [5]. Concurrently, micromachining by photolithography paved the way for research on capillary electrophoresis, marking the birth of single-phase microfluidics [6, 7]. Following the trend of utilizing smaller channels or

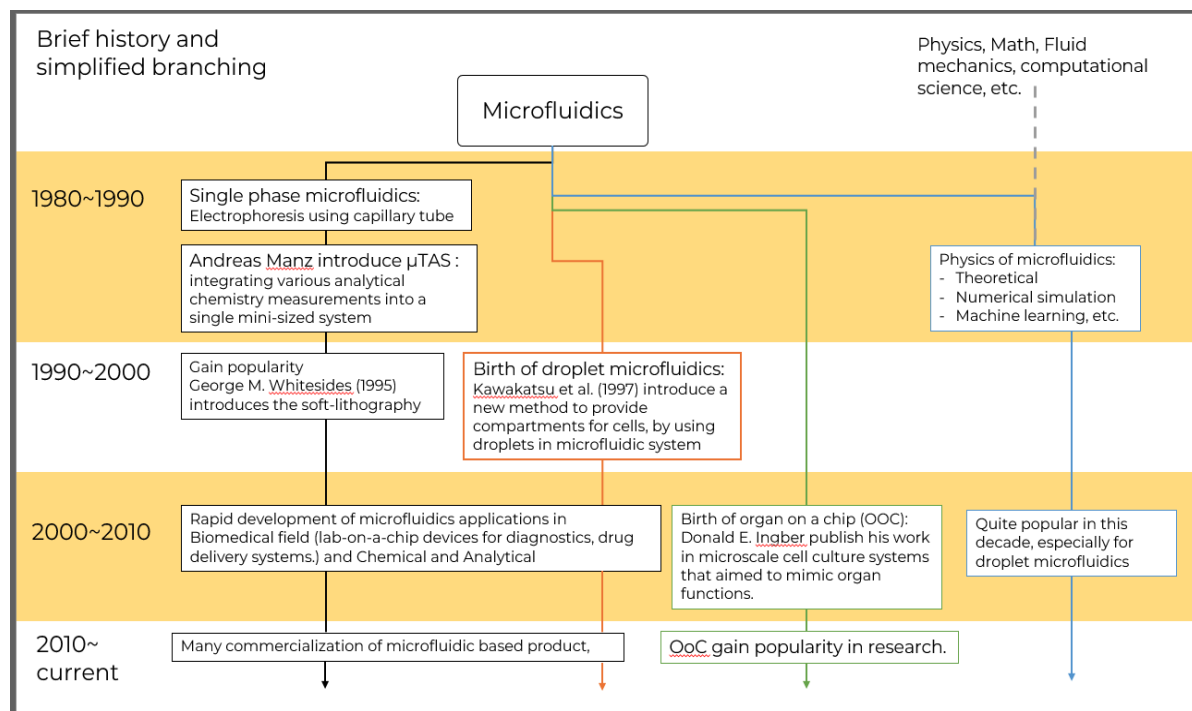


Fig. 1.1: Brief history of microfluidics

diameters for such processes, In 1990, Manz et al. promoted the concept of integrating various analytical chemistry measurements into a single mini-sized system [8]. The system is known as the miniaturized total chemical analysis system, μ TAS. The motivation of μ TAS was that these analytical chemistry methods were very useful; however, they are very time-consuming and require a fully equipped chemical laboratory and a qualified technician. Early development of μ TAS was mainly related to the miniaturization of chromatography [9], electrophoresis [10] and chemical detectors [11]. Integrating these microfabrication techniques with analytical chemistry spurred the initial demonstrations of applications in chemical analysis, biological assays, and environmental monitoring, establishing a foundation for the explosive growth of the microfluidics field in the following decades.

In the period between 1990 and 2000, the microfluidic field gained popularity after the introduction of the soft lithography technique by George M. Whitesides and colleagues [12]. The technique revolutionized the field by enabling the creation of microchannels in polymers, notably polydimethylsiloxane (PDMS), which allowed for easier and more versatile prototyping of microfluidic systems. The use of PDMS grants the integration of various components, such as valves and sensors, into the chip [13, 14]. The technique began to find numerous uses beyond capillary electrophoresis, such as polymerase chain reaction (PCR) [15], cell culture [16, 17] and separation [18], among other things.

In parallel, Kawakatsu et al. introduced a new method to provide microcompartments for cells by using droplets, giving birth to another branch of the field: droplet microfluidics [19]. The first generation of droplet generators includes the T-junction [20] and co-flowing of gas and liquids through an orifice, an initial flow-focusing type geometry [21]. This decade’s further work and innovations set the stage for microfluidics extensive applications in the field of biological [22], chemical [23], and medical research [19].

Between 2000 and 2010, the field of microfluidics saw significant advancements, particularly in the field of droplet microfluidics, which emerged as a powerful tool for a wide range of applications [24]. Breakthroughs during this period included the development of new techniques for generating, manipulating, and analyzing droplets with unprecedented precision and efficiency. Notably, the work by Anna et al. in 2003 introduced the “flow-focusing” microfluidic junction to produce monodisperse droplets with controlled sizes, paving the way for applications in chemical synthesis, biological assays, and materials science [25]. It is also the period when a high number of research focus on the fundamental aspect of droplet formation [26, 27, 28, 29]. A major breakthrough in the fundamental study of droplet formation particularly is shown by the work of Garstecki et al. in 2006 that introduced the scaling law for the droplet volume in the squeezing regime [30]. Remarkably, the period also saw the rise of biotechnology startups commercializing the microfluidic research results [31].

This period is also marked by the birth of another branch of microfluidic, namely the Organ on a chip (OoC). The first organ-level lung functions on a microfluidic chip were demonstrated by the group of Donald E. Ingber [32]. This field immediately became a popular research topic, as it can provide alternative platforms for drugs or vaccine testing [33].

From 2010 to the present, the field of microfluidics has continued to evolve rapidly, with significant breakthroughs in droplet microfluidics pushing the boundaries of applications in biology, chemistry, and medicine. Additionally, the field has seen significant progress in the development of integrated droplet microfluidic systems for clinical diagnostics and personalized medicine. Advances in the miniaturization of diagnostic assays within droplets have enabled rapid and sensitive detection of biomarkers and pathogens, promising faster and more accurate point-of-care testing. As a result, many companies currently provide droplet microfluidic techniques for biological research, particularly in the application of droplet microfluidic for single cell analysis [34].

1.2 Droplet Microfluidics

Droplet microfluidics, a specialized branch of microfluidics, has transformed biomedical applications by enabling precise, high-throughput techniques for single-cell analysis [36, 37], drug delivery [38], and point-of-care diagnostics [39]. This method leverages multiphase flow to create droplets—tiny, isolated compartments formed by immiscible fluids, typically oil and water—within microscale channels on a chip. Droplets range in size from a few microliters to nanoliters and act as miniature reactors capable of isolating and encapsulating cells, molecules, or nanoparticles for controlled experiments [40]. Their small volumes reduce reagent consumption, minimize waste, and allow rapid and efficient mixing of fluids for various chemical or biochemical processes [41, 35].

Microfluidic devices can generate and manipulate thousands of droplets per second with remarkable consistency and efficiency. These droplets facilitate complex operations such as mixing [42], merging [43], splitting [44, 45], sorting [46, 47], and trapping [48]. By combining these operations, advanced protocols such as on-demand concentration gradient generation [49] and droplet digital counting [50] are achievable, significantly expanding the scope of droplet microfluidics.

Droplet microfluidics is particularly valuable for applications like diagnostics, synthetic biology, and drug screening, where scalability, reproducibility, and sensitivity are critical. By reducing human intervention and enabling parallel processing, it supports advances in personalized medicine and lab-on-a-chip technologies. Additionally, the technique’s ability to generate high-throughput data makes it ideal for screening and analysis [51], further cementing its role as a cornerstone in modern biomedical research and applications.

At the heart of droplet microfluidic devices is a droplet generator, which has the role of producing the micro-sized volume of a liquid in another non-immiscible liquid. The most common

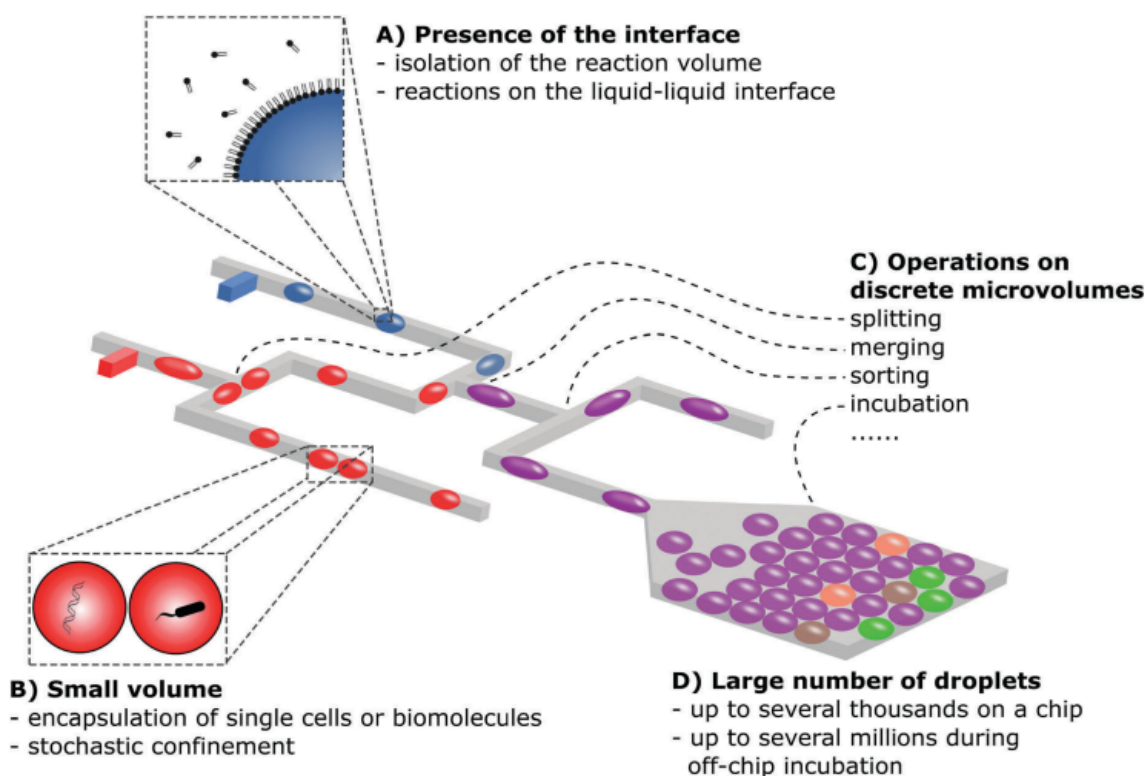


Fig. 1.2: Illustration of droplets inside a microfluidic network. The presence of an interface, small volume, flexible operations on droplets, and a large number of droplets gave the droplet microfluidic technique plenty of advantages. The image was adapted from [35].

type of droplet generator is the passive type droplet generator, in which droplets are generated based on the interaction between two (or more) immiscible fluids flowing through a junction of two or more channels. In the area of single-cell analysis, for example, microfluidic junctions such as co-flow, T-junction, and Flow-focusing are commonly used to produce monodisperse droplets with the size of few tens up to hundreds of microns as high-throughput cell culture experiment [52]. The use of microfluidic junctions requires a thorough mechanistic understanding of droplet formation; for example, it is important to understand how to tune the volume of the droplets for given flow conditions, channel geometries, and fluid properties. Despite the recent advances and the rapid adaptation of droplet microfluidic techniques in various fields, the mechanics behind droplet generation is a complicated phenomenon and has not been fully understood.

1.3 Motivation

The multiphase flow within a microfluidic channel is inherently complex. Given that the typical size of a microchannel ranges from a few tens to a few hundreds of micrometers, body forces such as gravity and inertia exert only a minor influence on the fluid flow. At this scale, the flow is predominantly governed by interfacial and viscous forces. Consequently, the flow behavior is typically characterized by the Capillary number, Ca , which represents the ratio of viscous forces to interfacial forces.

In systems involving two immiscible fluids, at least ten distinct flow patterns have been reported, arising solely from variations in the Capillary number [53]. The number of possible flow patterns increases when additional factors, such as the geometry of the microfluidic junction [29] and the wetting properties of the liquid-surface interface, are considered [28]. As a result, droplet microfluidic platforms that employ microfluidic junctions often operate within a limited range of Capillary numbers.

For instance, in a mono-dispersed droplet generation system designed for single-cell analysis, the operating range is typically confined to the low Ca regime, generally less than $\sim 10^{-1}$. This range corresponds to the *squeezing* regime of droplet formation, where interfacial forces dominate over viscous forces.

The squeezing droplet formation is a droplet-forming flow pattern that essentially consists of two key actions: 1) the blockage of the junction (and part of the downstream channel) by the droplet liquid followed by 2) the squeezing of the droplet liquid by the carrier fluid (or continuous phase liquid) [25, 26]. The squeezing action ends with the pinch-off of the neck of the droplet liquid, producing a droplet that moves downstream with the continuous phase. Without the blockage of the junction, there can be no squeezing action. Therefore, the resulting droplet always has a length larger than the channel width, which is the main characteristic of the squeezing regime. A scaling law was proposed for the squeezing droplet formation, which states that the resulting droplet volume is linearly proportional to the flow rate ratio between the two fluids, $l_D = c_1 + c_2 \left(\frac{Q_D}{Q_C} \right)$, where l_D is the dimensionless droplet volume, c_1 and c_2 are the constants with order of 1, and $\frac{Q_D}{Q_C}$ is the ratio between the rate of flow of the droplet liquid, Q_D , and the continuous phase, Q_C [30]. Strikingly, the scaling law does not include dependency of the resulting droplet volume to the fluid properties such as viscosity or interfacial tension, implying that the relative magnitude of the viscous shearing forces over the interfacial forces is unimportant. In other words, the flow is less sensitive to Ca . Consequently, the squeezing regime is the natural choice for the operating range of the droplet microfluidic system over other droplet-forming flow patterns, as it is less sensitive to fluid properties and to Ca over a wide operation range. It should be noted that droplet formation in the Ca range higher than of the squeezing regime has a different mechanism where both interfacial and the viscous forces play a role, commonly known as “dripping” [29] or “jetting” [28]. The resulting droplets are smaller than the width of the channel, and the mechanism is susceptible to the fluid properties and Ca .

While the scaling law for the squeezing regime has been experimentally validated in microfluidic T-junctions [30], flow-focusing [29] and cross-junction [54, 55], there seems to be more complexity in the process of squeezing droplet formation. Some previously reported measurements have shown some dependency of the droplet size on the Ca , showing that the squeezing droplet formation is not as simple as was proposed by the scaling law. A summary of the previous work on squeezing droplet formation is shown in Table 1.1.

There are conflicting reports regarding droplet formation in the squeezing regime. The experimental result of Cubaud and Mason [28], which includes measurement at a very low value of Ca ($\leq 10^{-3}$), shows there are two kinds of droplet formation within the squeezing regime: 1) the droplet formation has a strong dependency on Ca , i.e., $V_D \propto Ca^{-1}$ and 2) the droplet formation that almost independent to Ca , $V_D \propto Ca^{-0.17}$ (see Table 1). The threshold between the two droplet formations was proposed as $L_D/H \sim 2.5$. A similar threshold was also proposed in [55]. Indeed, there have been many reports that show the droplet volume in the squeezing regime being dependent on Ca with the proposed fitting equation more or less in the form of equation of $V_D \propto c_1 + c_2 \left(\frac{Q_D}{Q_C}\right)^k Ca^{-n}$, where c_1 , c_2 , k , and n as fitting parameters [56, 57, 58, 59]. In general, the reported values of k are approximately unity (1–1.2), whereas the values of n vary; however, they remain around or less than -0.3 (see Table 1.1). A seminal study by Korczyk et al. [60] has clarified this conflicting issue. Performing experiments using the T-junction at the very low Ca range, with Ca down to a few 10^{-5} , Korczyk et al. [60] introduced a new droplet formation regime which acts as the lower boundary of the squeezing regime (see last row in Table 1.1). The new regime is called the *leaking* regime, and it is characterized by the increase in droplet volume proportional to the Ca^{-1} . The name “leaking” comes from the effect that some part of the continuous phase leaks through the unoccupied space in the corner of the rectangular cross-section channel, the so-called gutter [61], and flows to the front of the droplet, instead contributing to the squeezing action. The leaking effect is negligible in the higher Ca , and the droplet formation can be considered independent of Ca , i.e., the squeezing regime. The low Ca droplet formation division into squeezing and leaking regimes explains the discrepancy between the previous reports. Curve fitting to the experimental data obtained in the low Ca range but not low enough may conclude the droplet formation which has weak dependency of Ca as it partially includes the transition between squeezing and leaking regime, for example in [56, 57, 58, 59], as shown in Table 1.1.

Table 1.1: Summary of the previous work related to the mathematical modeling of the droplet formation at low capillary number. Shown in the second row, the pioneering work of Garstecki et al. [30] introduced the squeezing regime droplet formation and its scaling law. Unless otherwise specified in the table, various mathematical symbols used in the previous literature were made consistent with the symbols used in the current text. W and H are the width and the height of a straight channel with a rectangular cross-section. Q_D and Q_C are the input flow rate of the droplet phase and continuous phase, respectively. Ca is the capillary number associated with the continuous phase. L_D and V_D are the dimensional length and volume of the droplet, respectively. l_D is the dimensionless droplet volume which is equal to L_D/W or $= V_D/HW^2$. A flow-focusing device is a cross-junction with an orifice. For simplicity, various constants or fitting parameters from previous studies are denoted as c_n , where the subscript n is used to differentiate between the constants.

Author	Model	Junction Types and Validation Method
Ward et al. [62]	$\frac{L_D}{W_{OF}} = 2.35 \left(\frac{Q_D}{Q_C}\right)^{0.25}$, where W_{OF} is the orifice width. Coefficients were obtained from curve fitting of the experimental data.	Flow-focusing. Experimental.

Author	Model	Junction Types and Validation Method
Garstecki et al. [30]	$l_D = 1 + c_1 \left(\frac{Q_D}{Q_C} \right)$, where c_1 is a constant in the order of unity.	T-junction. Experimental.
Cubaud and Mason [28]	$\frac{L_D}{H} = \begin{cases} 2.2 \times 10^{-3} (c_1 \text{Ca})^{-1} & : L_D > 2.5H \\ 0.5 (c_1 \text{Ca})^{-0.17} & : L_D < 2.5H \end{cases}$, where $c_1 = Q_C / (Q_C + Q_D)$.	Cross-junction Experimental.
Tan et al. [56]	$l_D = 1.59 \left(\frac{Q_C}{Q_D} \right)^{-0.20} \text{Ca}^{-0.20}$	Cross-junction. Experimental.
Lee et al. [29]	$\frac{L_D}{D_h} = \text{Ca}_1^{-0.33}$, where $\text{Ca}_1 = \frac{\mu_C Q_C W_D}{\gamma H \Delta Z} \left[\frac{1}{W_{OF}} - \frac{1}{2W_C} \right]$ with W_D , W_C and W_{OF} are the width of the droplet phase inlet, continuous phase inlet and orifice, respectively. ΔZ is the distance between the droplet phase inlet to the orifice. D_h is the hydrodynamic diameter of channel.	Flow-focusing. Experimental.
Fu et al. [58]	$l_D = \begin{cases} 0.72 \left(\frac{Q_C}{Q_D} \right)^{0.14} \text{Ca}^{-0.19} & : l_D \leq 2.35 \\ 0.30 \left(\frac{Q_C}{Q_D} \right)^{0.23} \text{Ca}^{-0.42} & : l_D > 2.35 \end{cases}$ Coefficients were obtained from curve fitting of the experimental data.	Cross-junction. Experimental.
Liu and Zhang [57]	$\frac{L_D}{W_C} = \left(c_1 + c_2 \frac{Q_D}{Q_C} \right) \text{Ca}^{c_3}$, where c_1 , c_2 and c_3 are fitting parameters with the values of 0.551, 0.277 and -0.292, respectively.	Cross-junction. Numerical Simulation.
van Steijn et al. [63]	$l_D = c_1 + c_2 \left(\frac{Q_D}{Q_C} \right)$. c_1 and c_2 were described as the droplet volume occupying the junction before the squeezing process and droplet volume behind the necking process. Both parameters were described as a function of geometrical parameters of the T-junction.	T-junction. Experimental.
Chen et al. [54]	$l_D = c_1 + c_2 \left(\frac{Q_D}{Q_C} \right)$. c_1 and c_2 were described as the droplet volume occupying the junction before the squeezing process and the volume of the continuous phase flowing to the junction and the gutter.	Cross-junction. Experimental.
van Loo et al. [55]	$l_D = \text{Ca}^{-c_1} \left(c_2 - c_3 \frac{Q_D}{Q_C} \right)$, where $c_1 \sim 0.20$, $c_2 \sim 0.05$, and $c_3 \in (0.40, 1)$ are the curve fitting parameters.	Cross-junction. Experimental.
Chen et al. [59]	$l_D = c_1 + c_2 \left(\frac{Q_D}{Q_C} \right)^{c_3} \text{Ca}^{c_4}$ c_1 , c_2 , c_3 and c_4 are the curve fitting parameters. $c_1 \in (-3.83, -0.02)$, $c_2 \in (0.37, 1.09)$, $c_3 \in (0.08, 0.14)$, and $c_4 \in (-0.24, -0.07)$.	Cross-junction. Numerical simulation.
Korczyk et al. [60]	$l_D = l_0 + v_{N0} \frac{Q_D}{Q_C} \left(1 - \frac{\beta}{q\text{Ca}} \right)$, where l_0 and v_{N0} are the dimensionless initial volume and the neck volume. β is a constant parameter defining the leaking strength.	Cross-junction. Experimental.

Although droplet formation in the low-Ca range has been clarified to involve two distinct regimes, it remains uncertain whether the same flow patterns apply across different junction geometries. Initial observations of droplet formation in a microfluidic cross-junction device show results similar to those in a microfluidic T-junction, specifically two distinct droplet formation modes: one sensitive to (Ca) and the other less so. I initially hypothesized that the governing principles of droplet formation in cross-junctions would mirror those in T-junctions. However, further investigation reveals a fundamental difference in the leaking flow behavior between these

geometries, as demonstrated through detailed experiments and mathematical modeling in the following sections.

This study proposes and experimentally validates a generalized scaling law for droplet size in cross-junction devices. The findings contribute valuable insights into droplet formation mechanisms under low-capillary conditions. Additionally, flow pattern maps for the low-Ca range are provided, highlighting unique flow patterns that emerge exclusively in cross-junction devices with high aspect-ratio channels.

The thesis also explores the application of droplets as cell incubators, presenting effective methods for cell delivery. I developed microfluidic chips with various construction and tested them to address the persistent issue of droplet evaporation. These advancements offer practical solutions for creating efficient droplet-based systems for cell culturing.

1.4 Aims

This study aims to investigate whether droplet formation at very low capillary numbers ($Ca \leq 10^{-3}$) in microfluidic cross-junctions follows the same mechanisms previously identified for T-junctions. The primary objective is to explore droplet formation in cross-junctions under various flow rate conditions and geometric configurations. Specifically, parameters that were previously considered constant will be reassessed to account for their Ca-dependency. The findings will lead to the development of a generalized scaling law, shedding light on the distinct droplet formation mechanisms associated with different junction geometries. Ultimately, these results will enhance our understanding of droplet formation in passive microfluidic junctions.

Another goal of this study is to evaluate the potential of using droplets as cell incubators. Three key challenges are addressed: the methodology for cell supply, the issue of droplet *per-vaporation*, and the survivability of cells within the droplets. The solutions to these challenges contribute as technical guidelines for conducting biological experiments using droplet microfluidic systems.

1.5 Hypothesis

It is hypothesized that droplet formation in a microfluidic cross-junction at very low Ca is significantly influenced by the Ca and differs notably from the mechanisms observed in T-junctions. This hypothesis is supported by preliminary experimental results, which indicate the formation of an elongated neck during droplet generation in the cross-junction device, which is not observed in the T-junction device. The elongation of this neck is hypothesized due to the surface tension effect and it is attributed to the geometry of the cross-junction, which allows for greater movement of the droplet along the main channel axis. Thus, it is expected that the neck elongation to exhibit a strong dependency on Ca.

1.6 Thesis Outline

The thesis consists of five chapters and is outlined as follows:

- **Chapter 1. Introduction** This chapter begins with an introduction to the field of microfluidics, outlining its significance and relevance to the research. A detailed discussion of the background motivates the study, highlighting gaps in the current understanding of droplet microfluidics. The chapter concludes by presenting the research objectives and the hypothesis that guide this work.
- **Chapter 2. Literature Review** This chapter provides an in-depth review of existing knowledge and previous studies relevant to this research. It begins with fundamental concepts, including viscous fluid flow and two-phase flow dynamics, which form the foundation

for understanding droplet behavior in microfluidic systems. The review then explores key advancements in the field, such as the emergence of flow patterns and droplet formation mechanisms. Special emphasis is placed on microfluidic droplet generators, including theoretical models and experimental observations of droplet formation. Additionally, the chapter discusses applications of microfluidic droplets, such as their use as cell incubators, and delves into the fundamental principles of droplet evaporation.

- **Chapter 3. Materials and Methods** This chapter describes the methodologies and experimental setups employed in the research. It begins by detailing two approaches to microfluidic device fabrication: CNC milling and photolithography. For each technique, the chapter provides an overview of the underlying principles, step-by-step procedures, and critical parameters, offering practical insights to streamline the learning process for future users. The section on photolithography includes technical notes aimed at optimizing the fabrication of microfluidic molds. The experimental setups for different aspects of the research are then presented. The first setup focuses on studying droplet formation under varying flow conditions, while the second investigates the use of droplets as cell incubators. The chapter also describes methods for supplying cells to droplets and addresses construction techniques for microfluidic chips designed to mitigate droplet evaporation. Finally, the measurement techniques used to analyze droplet formation, such as the image processing for detecting and measuring droplet size, are thoroughly explained.
- **Chapter 4. Results and Discussion** This chapter presents the research findings, starting with an overview of flow patterns observed in microfluidic cross-junctions under various flow conditions. At low capillary numbers (Ca), four distinct flow patterns are identified: "At Junction," "Downstream," "Parallel Flow," and the newly discovered "Bimodal" pattern. The chapter continues with a detailed analysis of droplet size measurements across different flow rate ratios and capillary number ranges. Classical squeezing scaling laws are revisited, discussing their limitations at low Ca values. A mathematical model is developed to extend the scaling law, incorporating capillary number dependency, to predict droplet sizes better. The latter part of the chapter addresses the use of droplets as cell incubators. A cell supply method that combines the pipette tip method and the negative pressure driving systems is shown to prevent cell clamping effectively, as demonstrated by the Poisson distribution of cells within the droplets. Various microfluidic chip architectures are tested to resolve the issue of droplet evaporation, with experimental results confirming the efficacy of the proposed designs. Finally, the survivability of cells inside droplets is evaluated, showing that the system is suitable for biological experiments.
- **Chapter 5. Conclusion** This chapter summarizes the key findings of the research, emphasizing their implications for the field of microfluidics. The significance of the contributions made to understanding droplet formation and their applications in biological experiments is discussed. The chapter concludes with recommendations for future research and potential areas for further development in droplet microfluidics.

Chapter 2

Literature Review

From a fluid mechanics perspective, droplet microfluidics is a multiphase flow in a network of microchannels. The droplet microfluidic may contain two or more streams of different fluids, with at least a pair of fluids that do not mix with each other, forming an interface. A continuous phase (CP) is a phase which occupies a continuously connected region of space inside the channel. CP acts as the host or the carrier fluid. The disperse phase (DP) is the phase which occupies disconnected regions of space inside the channel. The flow can be categorized as disperse flow if DP scatters in the host fluid. In contrary, the flow can be categorized as separated flow if there is a continuous stream of two or more fluids separated by interfaces. In droplet microfluidic, dispersed or separated flow may occur one after another along the channel, depending on the size of the droplets and the dimension of the channel. To avoid confusion with the term “disperse” while the flow is not always a disperse type, in this work DP is referred to as droplet phase instead of disperse phase.

2.1 Fundamentals

2.1.1 Single phase flow in a closed conduit

2.1.1.1 The continuum approximation

The continuum approximation assumes that a material is perfectly continuous. The assumption is valid in typical fluid mechanics experiments such as this work when a substance is viewed (or measured) on a much larger scale than the distance between atoms. On the other hand, a substance viewed at a very small scale shows the granularity of the molecules. Quantities measured on such a small scale, for example, the density or velocity of a point, may fluctuate strongly as illustrated in Fig. 2.1 [64]. The size of a point in the measurement should be large enough that it includes a large number of molecules, which is assumed to be homogeneously distributed, but not too large that the measurement cannot give the local value.

The work presented here was based on experimental measurements in flowing fluids at a relatively small scale that is observed under the magnification of a microscope. Most measurements were done from images with the highest spatial resolution of about 0.0175 mm/pixel. A point in this image, $1 \times 1 \text{ px}^2$, represents an area of fluids about $17.5 \times 17.5 \text{ }\mu\text{m}^2$ (approx. a circle with 20 μm of diameter), a size comparable to the cross-sectional of hot-wire anemometry. Does the measured quantity of a single point in an image satisfy the continuum assumption? At room temperature, a volume of $17.5 \times 17.5 \times 10 \text{ }\mu\text{m}^3$ contains approx. more than 3×10^{10} air molecules, enough amount to obtain the local average value of a point in space. In this case, the granular fluctuations of measured quantities due to molecular structure can be safely neglected. As such, the continuum approximation is valid in this work.

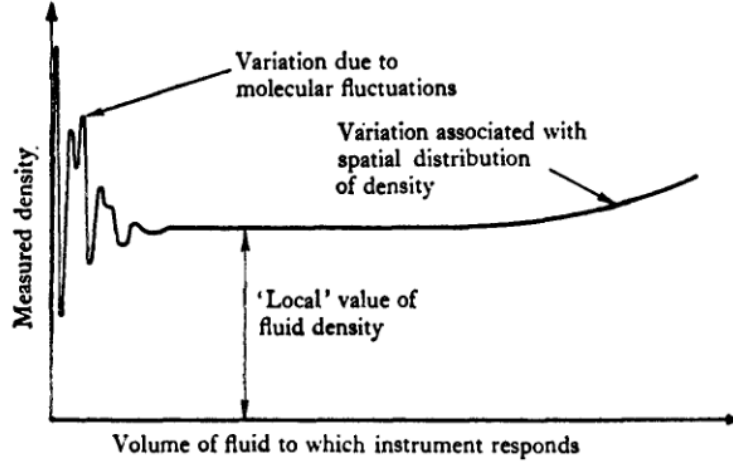


Fig. 2.1: Measured quantity variation due to the size of an instrument. In the volume where measured quantity fluctuation can be neglected, a fluid can be considered continuous. Image taken from Batchelor’s “An Introduction to Fluid Dynamics” [64]

2.1.1.2 Reynolds number and the viscous flow

Consider again a point or an arbitrary small element of fluid δV from a single-phase flowing fluid. This small element experiences pushing forces that cause motion, at the same time, feels the frictional forces from its surrounding, due to viscosity. Due to the viscosity effect, if the flow driving-force is taken away, the element will stop moving after some time. In general, all Newtonian fluid flow can be classified by the proportionality between the magnitude of inertia forces and the viscous forces, known as the Reynolds number:

$$\text{Re} = \frac{\rho U L}{\mu}, \quad (2.1)$$

where ρ and μ are the density and the dynamic viscosity of the fluid, respectively, whereas U and L are the characteristic velocity and length. In microfluidic flow, L is usually the diameter of the circular cross-section microchannel or hydraulic diameter, for example, $D_h = (2WH)/(W + H)$ for the rectangular cross-section microchannel.

Flow in a microfluidic channel is typically in the range of $\text{Re} \ll 10$. The low Re number comes from the low characteristic length L and velocity U relative to the viscosity value. The low Re flow is typically called viscous flow because of the strong viscosity effect that dominates the flow. Due to the strong viscous effect, the typical flow in a microfluidic channel is laminar, i.e., it does not allow for turbulence. This laminar flow can be seen easily from the experimental result of mixing between two miscible fluids at the Y-junction device [65]. Without turbulence, the mixing between two fluids occurs due to the diffusion effect only, which is slow.

2.1.1.3 Pressure-driven flow in a closed conduit

The viscous flow can be described by the Navier-Stokes equation. A steady and incompressible Navier-Stokes equation takes the form:

$$(\mathbf{v} \cdot \nabla) \mathbf{v} = \mathbf{g} - \frac{1}{\rho_0} \nabla p + \nu \nabla^2 \mathbf{v}. \quad (2.2)$$

Consider an elementary case, the flow inside a channel of finite length (L), with the vertical distance (h) much smaller than the width of the channel ($W \gg h$). Assume the velocity of the fluid as uni-directional in x-direction, $\mathbf{v} = (v_x(y), 0, 0)$ and the flow is driven by a constant pressure gradient ($-\Delta P/L$) in x-direction with negligible gravitational effect. The convective

term, $(\mathbf{v} \cdot \nabla)\mathbf{v}$, vanishes due to uni-directional flow and so the body force term, $\mathbf{g}$. Using the non-slip boundary condition, $\mathbf{v} = 0$ at $y = 0$ and $y = h$, the solution can be written as $v_x = \frac{\Delta P}{2\mu L}y(h - y)$, i.e., a parabolic velocity profile along the channel. The volumetric flow rate per width of the gap in a lateral direction is obtained by integration, $Q = \frac{h^3 \Delta P}{12\mu L}$. This solution is known as the plane Poiseuille flow.

In the three-dimensional case, the solution depends on the shape of the channel; for example, in the case of flow inside the cylindrical pipe, the solution — known as the Hagen-Poiseuille law — is written as [66]:

$$Q = \frac{\pi R^4 \Delta p}{8\mu L}. \quad (2.3)$$

In the case of the flow inside a rectangular channel with height H and width W , the solution is much more complicated [67]:

$$Q = \frac{\Delta P}{24\mu L}WH(W^2 + H^2) - \frac{8\Delta P}{\pi^5 \mu L} \sum_{n=1}^{\infty} \frac{1}{2n-1}^5 \left[W^4 \tanh\left(\frac{2n-1}{2W}\pi H\right) + H^4 \tanh\left(\frac{2n-1}{2H}\pi W\right) \right]. \quad (2.4)$$

All in all, the analysis resulting from the Navier-Stokes equation shows the proportionality of the resulting flow rate to the flow pressure drop and flow resistance, $Q \propto \frac{\Delta P}{R}$. The hydraulic resistance, R , represents the fluid flow resistance which depends on viscosity, μ , the length L , area A , and the channel shape. A general form of hydraulic resistance can be derived by dimensional analysis, written as:

$$R_{\text{hyd}} = \alpha \frac{\mu L}{A^2}. \quad (2.5)$$

α is a dimensionless parameter, which serves as a shape-correction factor[68].

2.1.1.4 Modeling the microfluidic circuit as a linear DC circuit

The linear relationship between the pressure drop, the flow rate, and the resistance in Hagen-Poiseuille's law resembles that of Ohm's law in the linear direct current (DC) circuit [69] (see Fig. 2.2). The pressure drop, the flow rate, and hydraulic resistance of the fluid flow are equivalent to the voltage difference (or simply voltage), current, and electrical resistance in the DC circuit, respectively. Consequently, a circuit of microfluidic channels can be treated mathematically in the same way as an electrical DC circuit analysis.

2.1.2 Multiphase flow in closed conduit

2.1.2.1 Surface tension

What is the surface of a liquid? A liquid is a condensed matter in which its molecules attract one another. A molecule in the midst of a bulk liquid has cohesive interactions with the neighboring molecule. On the other hand, a molecule that is on the surface loses half of its interactions; the liquid adjusts its shape to expose the smallest possible surface area. Accordingly, the surface tension, γ can be defined as:

- The amount of energy per unit area needed to increase a surface area by the amount of dA . The proportionality can be written as $\delta W = \gamma dA$, where γ has the unit of mJ/m^2 .
- The force per unit length needed to move the rod with fixed length, l as far as dx , as shown in Fig. 2.3. $4.dA = ldx$. The work needed to stretch the liquid film can be written as $\delta W = Fdx = 2\gamma ldx$. The factor of 2 reflects the presence of the two interfaces. In this

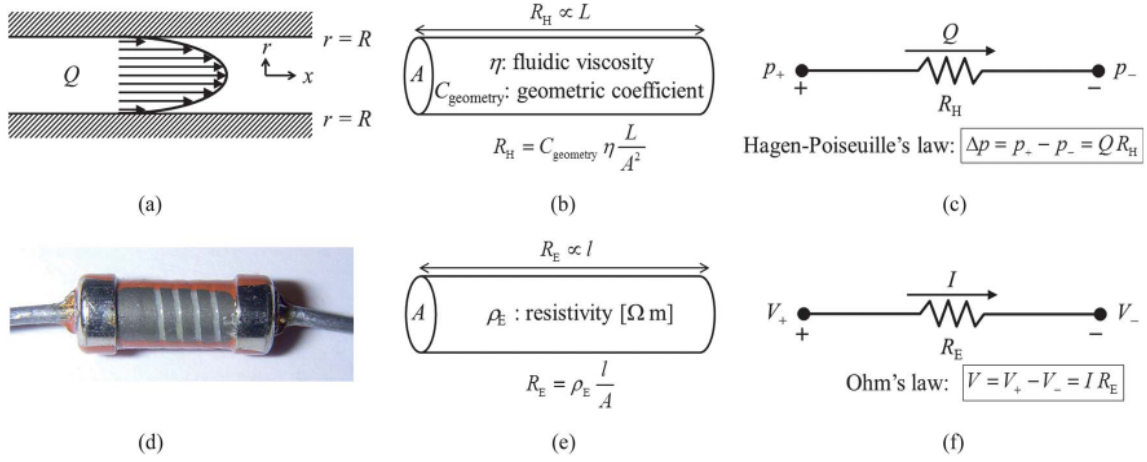


Fig. 2.2: The similarity between (a) fluid flow inside a channel and (b) electric current, (b) hydraulic resistance, and (e) electrical resistance. Consequently, (c) Hagen-Poiseuille's law in the fluid flow is analogous to (f) Ohm's law in the electrical DC circuit. Image is taken from [69].

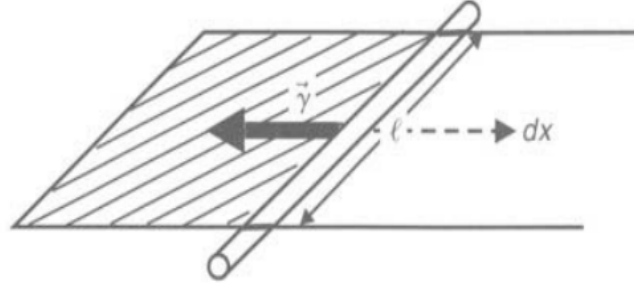


Fig. 2.3: Illustration of liquid film stretched by a rod as far as dx . If the rod is free to move, the surface tension force pulls back the rod to minimize the surface area. The image is taken from [70].

case γ has the unit of mN/m. Upon releasing of the force, the rod will move back to the original position due to surface tension forces.

2.1.2.2 Laplace pressure drop

Consider a water droplet immersed in oil. To minimize its surface area, the drop adopts a spherical shape of radius R . If the water-oil interface is displaced by an amount of dR , the work done by the pressure and capillary forces can be written as $\delta W = -P_i dV_i - P_o dV_o + \gamma dA$. p_i and p_o are the pressures inside and outside the droplet, respectively. γ is the interfacial tension of water-oil. The change of volume and surface area of the droplet is $dV_i = -dV_o = 4\pi R^2 dR$ and $dA = 8\pi R dR$, respectively. In the equilibrium state, $\delta W = 0$, such that:

$$\begin{aligned} p_i - p_o (4\pi R^2 dR) &= \gamma 8\pi R dR \\ p_i - p_o &= 2 \frac{\gamma}{R} \end{aligned} \quad (2.6)$$

Here, Eq. 2.6 shows that the pressure jumps across the droplet interface is proportional to γ/R , which means the smaller the radius, the larger the pressure difference. The increase of hydrostatic pressure at an exact location across an interface is equal to the product of the surface

tension and the curvature of the interface (κ), known as Young-Laplace law, written as:

$$\Delta p = \gamma \kappa = \gamma \left(\frac{1}{R} + \frac{1}{R'} \right). \quad (2.7)$$

R and R' are the radii of curvature of the interface at an exact location.

2.1.2.3 Wetting of the surface

When a drop of liquid is placed down on a flat surface, the drop may spread on the surface (e.g., water drop on a clean glass surface) or remain in one place with a spherical cap (e.g., water drop on a plastic surface). The first condition is known as total wetting, while the latter is known as partial wetting. The wetting condition can be described by spreading parameter,

$$S = \gamma_{SO} - (\gamma_{SL} + \gamma), \quad (2.8)$$

where γ_{SO} , γ_{SL} , and γ are the surface tension of solid-air, solid-liquid, and liquid-air, respectively. If $S > 0$, the liquid spreads completely, and the contact angle, $\theta_E = 0$. When $S < 0$, the drop forms a spherical shape with $\theta_E > 0$. If $\theta_E < 90^\circ$, it corresponds to a “mostly wetting” state, also known as hydrophilic (for water) or oleophilic (for oil). In contrast, $\theta_E > 90^\circ$ indicates the non-wetting state, known as hydrophobic (for water) or oleophobic (for oil). Another relation can be formulated by projecting the equilibrium forces in the contact point onto the solid plane, known as Young’s law: $\gamma \cos \theta_E = \gamma_{SO} - \gamma_{SL}$. Such that S can be written as $S = \gamma (\cos \theta_E - 1)$.

2.1.2.4 Role of surfactants

A surfactant is a molecule that consists of two parts: the part that likes to mix with water, “hydrophilic” and the parts that rejects water, “hydrophobic”. In general, the hydrophobic part is formed by one or multiple aliphatic compound chains, in most cases $\text{CH}_3(\text{CH}_2)_n$. The hydrophilic part is usually an ion, either anion or cation, which forms a polar head. The polar head likes liquids with a high dielectric constant, such as water. The hydrophilic part can also consist of short chains of neutral units that are soluble in water, e.g., polyethylene oxide. Commonly, there are dozens of carbon atoms in the aliphatic part and five or six ethylene oxide groups in the hydrophilic part.

In the case of water droplets in oil, as in the typical droplet-based microfluidics, the hydrophilic part of the surfactants is attached to the water droplet while the hydrophobic part sticks out, forming a thin hydrophobic layer covering the droplet. This hydrophobic layer offers two benefits: It prevents the water droplet from wetting the microchannel surface, and it prevents the coalescence of droplets.

2.1.2.5 Important dimensionless number in microfluidics

Apart from the aforementioned Reynolds number, there are other dimensionless numbers that are important in the field of microfluidics, as listed below. U and L are the characteristic speed and length of the flow, respectively. μ , ρ , and γ are the viscosity, density, and surface tension of the fluid, respectively. $\Delta\rho$ is the density difference between two fluids. g is the gravitational constant.

1. The capillary number, Ca , is a dimensionless number that compares the magnitude of viscous forces and surface tension forces. It can be written as

$$Ca = \frac{\mu U}{\gamma} \quad (2.9)$$

2. The Weber number, We , is a dimensionless number that compares the inertia forces to the surface tension effect.

$$We = \frac{\rho U^2 L}{\gamma} \quad (2.10)$$

3. The Bond number, Bo , is a dimensionless number that compares the magnitude of gravitational forces and surface tension.

$$Bo = \frac{\Delta \rho g L^2}{\gamma} \quad (2.11)$$

2.1.2.6 The flow field with dispersed droplets

The flow field can be divided into two categories: dispersed droplet flows, where droplets are smaller than the channel width, and slug flows, where the droplets occupy most of the channel's cross-section. In the dispersed droplet flows, droplets flow with the local velocity of the fluids, i.e., droplets move as particles move with the flow of the host fluids. Thus, the dispersed droplet flows, except for the droplet that is located near a solid boundary, behave as a single-phase viscous flow. The pressure drop of the flow can be approximated as previously described in the Hagen-Poiseuille flow.

The slug flows are much more complex. Let's consider a flow in a straight microfluidic channel, with a single or trains of droplets confined inside the channel. If the droplet phase is a gas, the flow is commonly known as Taylor flow. In fact, the initial study on this subject was focused on the motion of gas bubbles in a capillary [71]. Regardless of which fluid phase is used as the droplet, the droplet phase should not wet the wall, and the continuous phase completely wet the wall. With such conditions surrounding the droplet, a thin liquid film exists. It is an intriguing fluid flow problem. The flow is driven by the pressure difference between the two ends of the channel, resulting in an average velocity of $U_{\text{ext}} = Q_{\text{total}}/A$, where Q_{T} and A are the total flow rate and the cross-section area, respectively. A droplet may move at the same, higher, or lower speed as the mean velocity speed. The thin film thickness and velocity may vary, and so the droplet shape. In general, the flow depends on the viscosity ratio between CP and DP, the Capillary number of the flow, the geometry of the microchannel, and the interfacial tension.

In a capillary tube with a circular cross-section, the droplet fully occupies the circular channel. However, there is a thin film of the continuous phase lubricating the droplet and connecting the plug between droplets (see Fig. 2.4). Bretherton [71] proposed a scaling law for the thickness of the liquid film as the function of the Capillary number of the droplet phase, $e/H \propto Ca_D^{2/3}$ with $Ca_D = \mu_C U_D / \gamma$. It is understood that due to the lubrication film, the droplet moves faster than the mean velocity of the total flow rate. Naturally, the speed difference is proportional to the thickness of the liquid film, such that [72, 73]:

$$\frac{(U_D - U_{\text{ext}})}{U_D} \propto Ca_D^{2/3}. \quad (2.12)$$

In the channel with a rectangular cross-section, droplets do not fill the channel completely. Some parts of the continuous phase may flow through the gutter (see Fig. 2.4). Thus, the flow is much more complicated. The interfacial tension, the viscosity ratio, and the flow rate determine the shape and motion of the droplets. Wong et al. [61] argue that the droplet acts as a leaking piston such that there is a leaking flow through the gutter, and the droplet moves slower than the mean inlet velocity. The speed difference is inversely proportional to Ca [74] as:

$$\frac{(U_D - U_{\text{ext}})}{U_D} \propto -Ca^{-1/3} \quad (2.13)$$

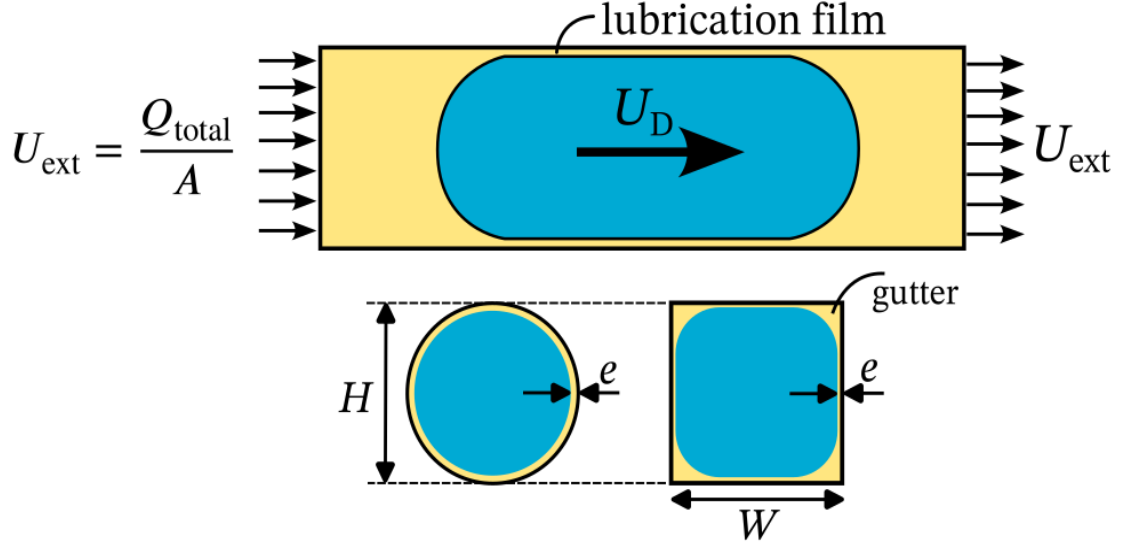


Fig. 2.4: Droplet may move at equal, higher, or lower speed than the mean velocity inside the microfluidic channel.

2.2 Microfluidic droplet generators

2.2.1 Active droplet generator

Active droplet generators in microfluidics are advanced droplet generators that are equipped with additional element(s) to control the droplet formation with high precision. Unlike passive droplet generation methods, which rely solely on the inherent properties of the fluids and the flow conditions, active droplet generators introduce external energy to influence droplet formation. This external energy can be supplied in various forms, including electrical, magnetic, centrifugal, optical, thermal, and mechanical forces. These active techniques offer more precise control over droplet size, distribution, and production rates, enabling either fine-tunable or on-demand droplet generation. The active droplet generator is crucial for applications such as droplet production in highly viscous fluids or working under conditions where passive methods might fail, such as in systems with ultra-low interfacial tension [75].

There are several types of active droplet generators, each categorized based on the nature of the external force applied. Electrical methods, for example, employ electric fields to alter the interfacial tension or induce droplet deformation and detachment. Magnetic techniques use magnetic fields to manipulate droplets containing magnetic particles or fluids with magnetic susceptibility. Centrifugal methods utilize rotational forces to generate and control droplets, which are particularly useful in portable lab-on-a-chip devices. Optical methods use focused laser beams to manipulate droplets by inducing localized heating or through radiation pressure. Thermal methods control droplet formation by selectively heating portions of the microfluidic channel, which changes the interfacial tension or viscosity of the fluids involved. Mechanical methods involve the physical deformation of the microfluidic channel or direct mechanical actuation to generate droplets [76]. Each active method provides distinct advantages, such as faster response times, handling a broader range of fluid properties, and the potential for more complex droplet manipulation scenarios.

2.2.2 Passive droplet generator and the microfluidic junctions

Passive droplet generation is a droplet formation process without the help of active elements. The formation process relies on several factors: fluid flow condition, the geometry of the mi-

crofluidic channel, and the properties of the fluids. As shown in Fig. 2.5, the passive droplet generation methods can be divided into two categories [77]:

- Hydrodynamic Capillary Traps.** Hydrodynamic capillary traps are microfeatures, like microwells, used in microfluidic devices to create and capture droplets through hydrodynamic forces. In these devices, the dispersed phase forms droplets when they enter the microfeatures, driven by capillary forces. Typically, this involves filling both a microfluidic channel and its wells with an aqueous solution and then introducing an immiscible liquid, such as oil, to replace the aqueous phase in the channel while leaving it in the wells. This action breaks the continuous liquid into discrete droplets that are trapped within the microwells, forming droplet arrays (see Fig. 2.5a). Another approach utilizing capillary traps is the SlipChip, a microfluidic device engineered for precise droplet generation and manipulation through a sliding mechanism (see Fig. 2.5b). The SlipChip comprises two glass plates separated by an oil layer, with one plate containing wells and the other channels. As these plates slide against each other, the continuous fluid path is interrupted, generating droplets that are captured in the wells, thus forming a droplet array.
- Hydrodynamic junctions.** A hydrodynamic junction (or microfluidic junction) is a droplet generator that typically involves the confluence of two immiscible fluids, where one fluid (dispersed phase) is introduced into another (continuous phase) at a specific junction, leading to the formation of droplets. The breakup of the droplet phase into droplets is due to the forces exerted by the continuous phase. The droplet formation process at these junctions is governed by the interplay of several forces, including viscous drag, interfacial tension, and inertial forces, which work together to determine the size, shape, and uniformity of the droplets.

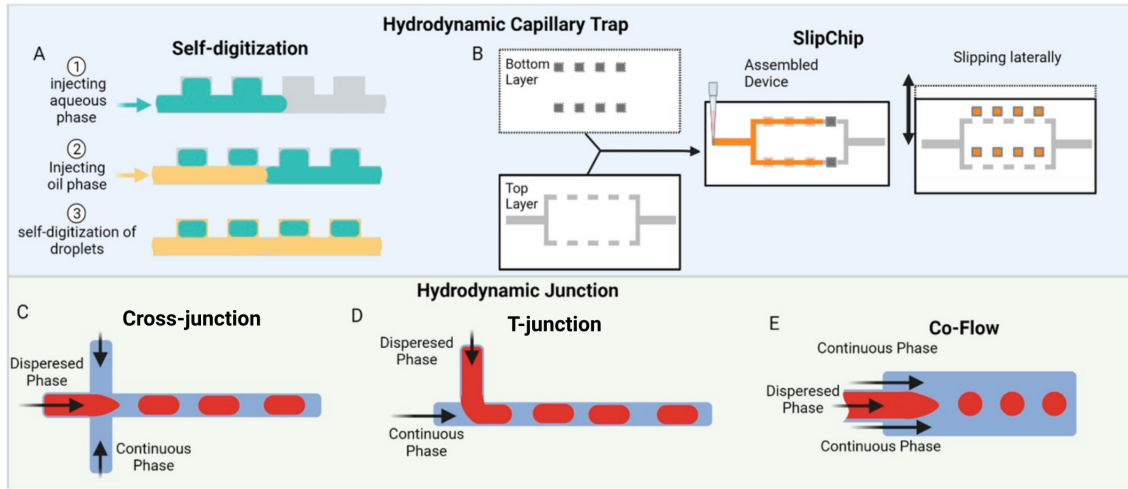


Fig. 2.5: Various passive droplet generators which can be grouped based on its mechanisms: 1) droplet formation based on hydrodynamic trap such as (a) self-digitization and (b) SlipChip; 2) droplet formation based on various hydrodynamic junction such as (c) Cross-junction, (d) T-junction and (e) co-flow. The image was adapted from [52].

Several types of hydrodynamic junctions are used for droplet generation, each with unique characteristics and applications. The most common types are:

- T-Junctions:** In T-junctions (Fig. 2.5d), the dispersed phase is introduced perpendicularly into the continuous phase, often at a T-shaped or Y-shaped intersection. The continuous phase flows along the main channel and exerts shear forces on the dispersed phase as it enters from the side channel. Combined with the pressure differential across

the junction, these shear forces cause the dispersed fluid to pinch off and form droplets. The size of the droplets in T-junctions can be precisely controlled by adjusting the flow rate ratio between the two phases. T-junctions are widely used due to their simplicity and ability to produce highly monodisperse droplets.

2. **Co-Flow:** Co-flow junctions (Fig. 2.5e) involve parallel streams of the continuous and droplet phases flowing together through a narrow channel. The dispersed phase is injected into the center of the channel, surrounded by the continuous phase, and droplets are formed as the dispersed phase jet destabilizes under the influence of interfacial tension and viscous forces. Co-flow systems are particularly useful for producing droplets with smaller sizes and are often employed in applications requiring high precision and control over droplet size. Droplets generated in co-flow configurations are typically more monodisperse when formed in the dripping regime compared to the jetting regime, where they become polydisperse.
3. **Cross-Junctions:** Cross-junctions (Fig. 2.5c) refine the principles of T-junction by introducing another continuous phase channel from opposite sides to squeeze the droplet phase from both sides. Another common variant of the cross-junction is the flow-focusing device, which utilizes a narrow orifice at the end of the junction [26, 25]. The flow-focusing device is effective in generating smaller droplets, typically with diameters significantly smaller than the orifice itself, making them ideal for applications requiring nano-sized droplets or emulsions.

The working principle across these junctions revolves around the balance of forces acting on the interface between the two fluids. In low-capillary-number regimes (characteristic of squeezing or dripping modes), viscous shear forces and interfacial tension dominate, causing the dispersed phase to break into droplets once the pressure difference overcomes the interfacial tension. In higher capillary-number regimes, inertial forces can also play a significant role, particularly in jetting modes, where the fluid inertia causes the dispersed phase to stretch into a jet that subsequently breaks into droplets due to Rayleigh-Plateau instability.

2.3 Flow patterns

When two immiscible liquids pass through microfluidic junctions, the resulting flow patterns vary based on factors such as flow conditions, fluid properties, and the geometry of the junctions. Moreover, the wetting condition of the surface also largely affects the generated flow patterns. Flow patterns generally arise from the interplay of different forces, including surface, viscous, and inertial forces. Given the small scale of the channels, the gravitational forces are minimal and, therefore, can be neglected.

2.3.1 General flow patterns in microfluidic junctions

As there are various forces affecting the produced flow patterns, there are at least five forces interacting together during the process of droplet formation: each liquid viscous and inertial forces and the interfacial forces. For decades, researchers have been working to find all the flow patterns and have been trying to generate the general flow patterns map, which used various dimensionless numbers, i.e., to reduce the dimensionality of the problem. It is, however, not a simple task as the phenomenon is very complicated. For example, the flow pattern generated by liquid pairs of high viscosity ratio can be very different compared to the flow patterns typically generated in moderate viscosity ratio [78]. It is no coincidence that the flow pattern map plotted by using composite dimensionless numbers (a combination of dimensionless numbers) provides the best view of the flow pattern map. The most recent, which is supposed to be the complete flow pattern map, is provided by Yao et al. [53], which is shown in Fig. 2.6.

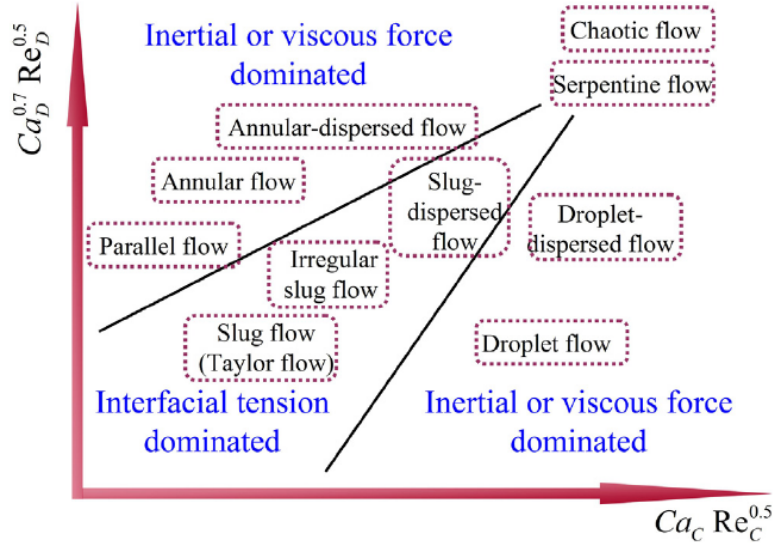


Fig. 2.6: The flow patterns in junction type droplet generator. The image was taken from [53].

2.3.2 Flow patterns based on varying Capillary number

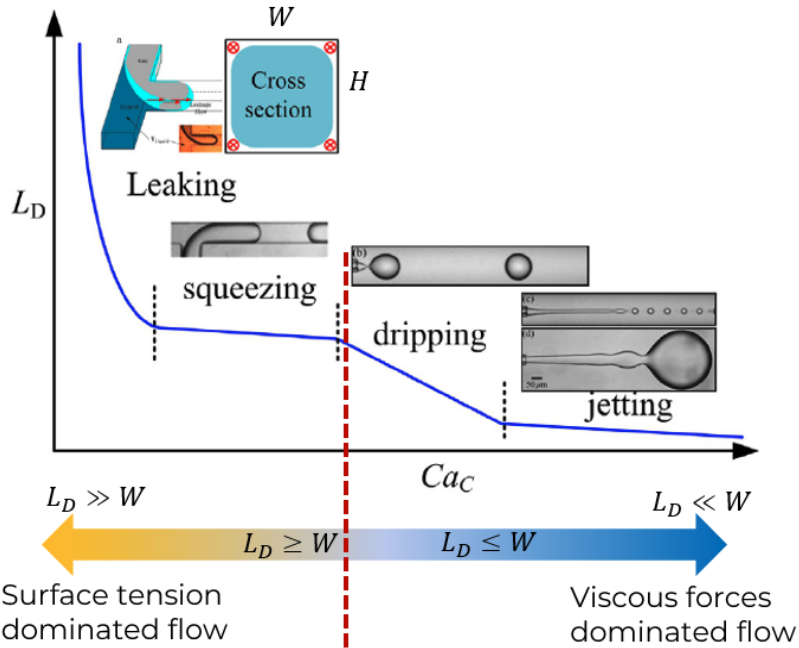


Fig. 2.7: Various flow patterns emerge by changing the capillary number while setting the flow ratio to be constant. The image was taken from [53].

Another interesting flow pattern map is not based on the composite dimensionless number, as shown previously. It is rather a subset of the previous flow patterns map, and it is based only on the varying Capillary number of the continuous phase (Ca). From the experimental side, it is simple: keep the flow rate ratio constant and vary the flow rate of the continuous phase only. Then observed the phenomena and measured the droplet size (see Fig. 2.7). Apparently, the flow pattern based on varying (Ca) shows a general pattern across different liquid pairs and different geometry, which is illustrated in Fig. 2.7. In low (Ca), the flow is surface tension dominated, while in high (Ca), the flow is viscous force dominated. There are typically four flow patterns that can be deduced from this kind of observation: Leaking, Squeezing, Dripping, and

Jetting. In surface tension-dominated flows, such as in the squeezing and leaking regime, the droplet size is larger than the channel width. In the viscous force-dominated flow, such as in dripping and jetting regimes, the droplet size is smaller than the channel width. The four flow patterns shown as in Fig. 2.7 are typical across various liquid pairs of moderate viscosity ratio and across different microfluidic junctions. Thus, this kind of map, although it does not have complete information as compared to the previous map with composite dimensionless numbers, can provide practical information for users about the operating range of droplet formation. Commonly, the production of monodisperse droplets for biological experiments is performed in the squeezing regime. As shown in Fig. 2.7, the produced droplet size in the squeezing regime is almost constant over a wide range of Ca , which ensures the production of mono-disperse droplet size despite the unavoidable experimental flow fluctuation.

2.4 The scaling law for the droplet volume

2.4.1 The squeezing regime of droplet formation

The squeezing mechanism of droplet formation in microfluidic junctions occurs at low capillary numbers, where interfacial forces dominate over shear stresses. In this flow regime, the breakup of droplets or bubbles is primarily driven by the pressure drop across the forming droplet rather than by the shear forces exerted by the flowing continuous phase.

As an example, in a T-junction (Fig. 2.8a, the CP flows through the main channel, while the DP enters from a perpendicular inlet. As the DP penetrates into the main channel, it begins to form a droplet. When the droplet nearly fills the cross-section of the main channel, it creates significant resistance to the flow of the continuous phase, leading to a pressure buildup upstream of the droplet. This increased pressure results in the squeezing of the neck of the dispersed phase, which eventually leads to droplet pinch-off. The mechanism of squeezing droplet formation is similar across different microfluidic junctions. Generally, the process can be divided into three distinct stages: Filling, Necking, and Pinch-off (see Fig. 2.8).

During the filling stage, the DP enters the junction and grows until it fills most of the junction. At this point, the DP occupies a significant portion of the channel cross-section, which initiates the buildup of pressure upstream as the CP tries to flow past the forming droplet. The filling stage ends when the CP is able to deform and push the DP downstream from the junction.

In the necking stage, CP squeezes the forming droplet as it tries to force its way through the narrowed space. The squeezing of the forming droplet creates a “neck,” which progressively gets thinner. The rate of necking is closely related to the flow rate of the CP, which determines how quickly the neck is squeezed.

The pinch-off stage occurs when the neck of the neck becomes sufficiently thin, i.e., when the neck width is close to the channel’s height, it can no longer sustain the droplet’s connection to the inlet. The interfacial forces cause the neck to rupture, leading to the detachment of the droplet. The pinch-off is a rapid process, concluding the droplet formation sequence.

The size and frequency of the resulting droplets are influenced by both the flow rates of the CP and DP, with the CP’s rate dictating the speed of necking and the DP’s rate controlling the growth of the droplet. Droplet volume can be calculated as follows: during the end of the filling stage, there is already some volume of DP downstream of the junction, V_0 . Then the additional volume comes from the DP flow rate, Q_D during the necking process, such as $Q_D\tau$ where τ is the duration of necking. Thus, a simple scaling relation describing the size of the droplets formed under the squeezing mechanism can be written as [30]:

$$V_D = V_0 + Q_D\tau, \quad (2.14)$$

where V_D is the droplet volume. There are few assumptions that make Fig. 2.12 valid in the squeezing regime:

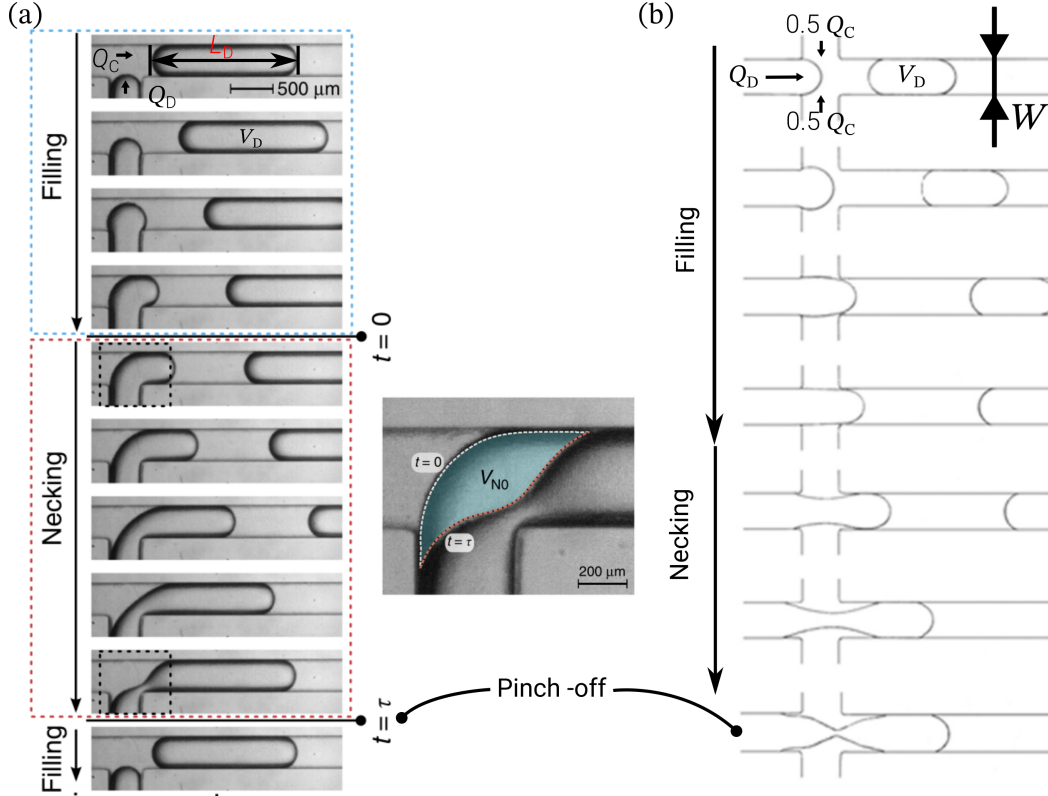


Fig. 2.8: Image sequence of squeezing droplet formation in (a) T-junction and (b) cross-junction. The inset shows the total displaced DP volume because of the necking process, V_{N0} . The images in a) and b) were adapted from [60] and [54], respectively.

1. The initial volume V_0 does not depend on the flow condition nor the properties of the fluid, which makes it a constant but has a dependency on the junction geometry [63].
2. During necking, all CP are flowing to the necking area which displaces a total volume of DP, V_{N0} until the pinch-off with the rate of Q_C . Thus, the necking time can be defined as $\tau = V_{N0}/Q_C$.
3. The flow conditions do not affect the magnitude of V_{N0} , which means V_{N0} is a constant parameter that may have dependency on the junction geometry.

These assumptions are valid in the squeezing regime, and it has been verified experimentally[63]. Using the above assumptions, Eq. 2.14 can be written as:

$$V_D = V_0 + V_{N0} \frac{Q_D}{Q_C} \quad (2.15)$$

Eq. 2.15 indicates that the droplet size depends on the flow rate ratio of the two phases, but it is independent of their properties: viscosities and interfacial tension. It is so because the necking does not depend on the generation of thin liquid filament, which requires high shearing force as in the higher Ca droplet formation mechanism. The necking is permitted due to the DP blocking the downstream channel. The blockage causes CP accumulation in the junction, igniting the necking process. Thus, the squeezing droplet formation is less sensitive to the fluid properties and very much dependent on the junction geometry. The stability of squeezing droplet formation makes it highly suitable for applications that require the production of monodisperse droplets. Eq. 2.15 can be written in dimensionless form by using dimensionless quantities such as $l_D = V_D/(HW^2)$, $l_0 = V_0/(HW^2)$, $v_{N0} = V_{N0}/(HW^2)$ and $q = Q_D/Q_C$:

$$l_D = l_0 + v_{N0} q \quad (2.16)$$

l_D can also be understood as dimensionless droplet length $l_D = L_D/W$.

2.4.2 The leaking regime in the T-junction

The leaking regime in droplet formation emerges at very low Ca , much lower than of the squeezing regime (see Fig. 2.7). This regime challenges the conventional understanding provided by the squeezing regime models, i.e., in the low Ca , the droplet formation is insensitive to the flow conditions, as previously discussed. As shown previously in Fig. 2.7, the leaking regime was observed experimentally in microfluidic T-junctions where there was an abrupt increase in droplet length following a decreasing Ca . At very low capillary numbers, the CP no longer effectively squeezes the forming droplet, and a significant portion of it leaks around the droplet through the corners of the channel—which are not filled by the droplet due to its non-wetting nature against the channel walls—the so-called gutters [61, 79]. This leakage alters the droplet formation dynamics, leading to a strong dependence of droplet size on the Ca , which is a hallmark of the leaking regime.

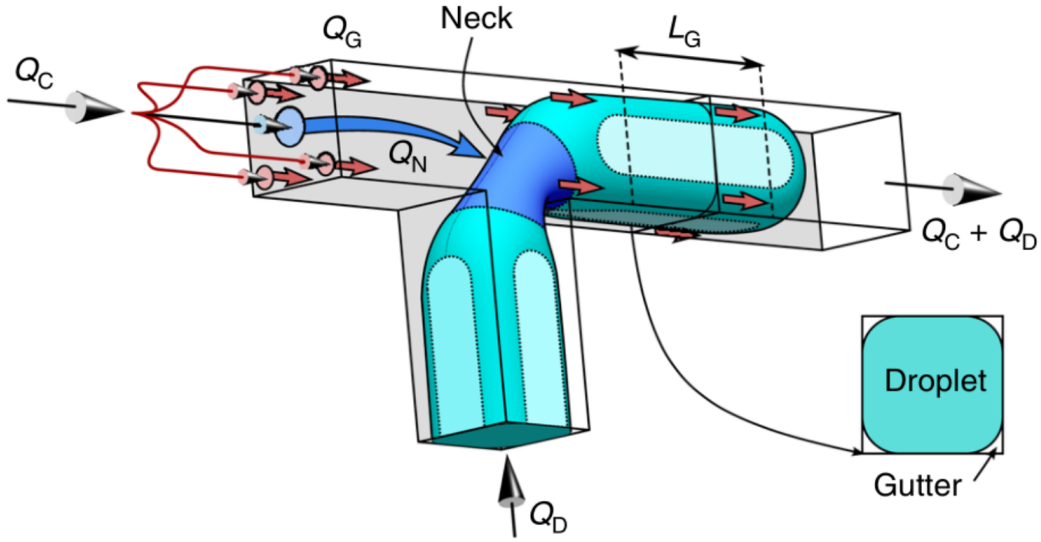


Fig. 2.9: Illustration of necking flow, Q_N ; gutter flow, Q_G during droplet formation and the gutter. Bypass flow is the total of all gutter flow $Q_B = 4Q_G$. Image was adapted from [60]

It is understood that droplets in a rectangular microchannel behave as leaking pistons, the original squeezing model assumes negligible CP flowing through the gutters. However, in the leaking regime, the assumption fails. Instead, the model accounts for the CP leaking around the droplet through the gutters,

Korczyk et al. [60] developed a mathematical model that explains the leaking droplet formation in a T-junction device. The leaking model assumed that not all of the CP flow (with the rate of Q_C) contributes to the necking. Instead, only some fraction of the CP flow contributes to the necking, with the rate of Q_N . The rest of the CP flow bypasses the forming droplet through the gutter with the rate of Q_B , as shown in Fig. 2.9). Based on this assumption, Eq. 2.16 was modified to include the leaking effect:

$$l_D = l_0 + v_{N0}q(1 + \bar{\eta}), \quad (2.17)$$

where η is the ratio between the rate of bypassing flow and necking low, $\eta = Q_B/Q_N$. $\bar{\eta}$ denotes the time-averaged η . Korczyk et al. [60] have found the solution for η by using DC electrical circuit analogy and proposed the new scaling law that includes the leaking regime as:

$$l_D = l_0 + v_{N0} q \left(1 + \frac{\beta}{qCa} \right), \quad (2.18)$$

where β is a constant parameter consisting of the geometrical factor of the gutter [60]. Eq. 2.18 states that the l_D is inversely proportional to Ca in the very low Ca regime. Whereas for higher Ca regime, the last term of the Eq. 2.18 diminishes, and the model returns to the original squeezing model (Eq. 2.16).

2.5 Droplets for cell isolation and incubation

2.5.1 Single-cell analysis

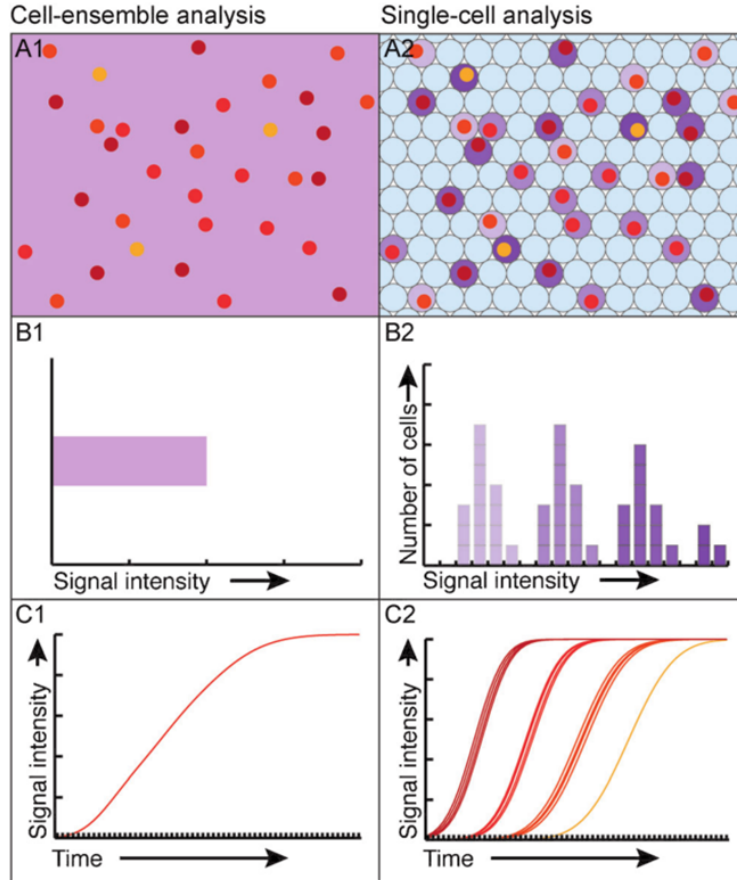


Fig. 2.10: Comparison between ensemble and single-cell analysis. The image was taken from [80].

Droplet microfluidics enables the compartmentalization of single cells into tiny droplets, each serving as an isolated microenvironment where cellular processes can be monitored precisely. The benefits of droplet microfluidics in biological research are vast, including the ability to analyze large populations of cells at the single-cell level, thus providing insights into cellular heterogeneity, which is often masked in traditional ensemble analysis. Comparison between Cell-Ensemble Analysis (traditional) and Single-Cell Analysis, as illustrated in Fig. 2.10, are summarized as below:

1. **Revealing Cellular Heterogeneity:** Among genetically identical cells, significant differences can exist in gene expression, size, growth rates, and other characteristics. Traditional

cell-ensemble analysis, which typically provides average values of the population, often masked these differences.

2. **Identification of Rare Cell Types:** By isolating the cells inside the droplet and analyzing it individually (or in small numbers) it is possible to detect and characterize rare populations, for example, stem cells, immune cells with unique roles, or cancer cells with distinct mutations,
3. **Accurate Representation of Population Dynamics:** Ensemble analysis assumes that the population dynamics follow a normal distribution, which may not always be true. Single-cell analysis, however, can reveal complex, non-Gaussian distributions of traits within a population, such as multimodal distributions or other non-standard patterns. This level of detail provides a more accurate representation of the true biological variability within a cell population, allowing for more precise modeling and predictions of cellular behavior (See Fig. 2.10).
4. **Enhanced Sensitivity and Specificity:** By isolating single cells, droplet microfluidics allows for the amplification and detection of low-abundance signals, such as rare transcripts or proteins, which might be diluted or undetectable in bulk analysis. This enhanced sensitivity is crucial for the accurate detection and quantification of biomarkers, especially in early disease diagnosis or in monitoring subtle cellular responses to treatments.
5. **Dynamic Studies and Temporal Analysis:** Single-cell analysis allows tracking of individual cell changes over time. It can provide insights into dynamic processes such as cell differentiation, response to stimuli, and cell cycle progression. Traditional ensemble methods do not easily accommodate such temporal studies at the single-cell level, often missing critical information about how cells evolve or respond under different conditions.

2.5.2 Cell culture methods in droplets

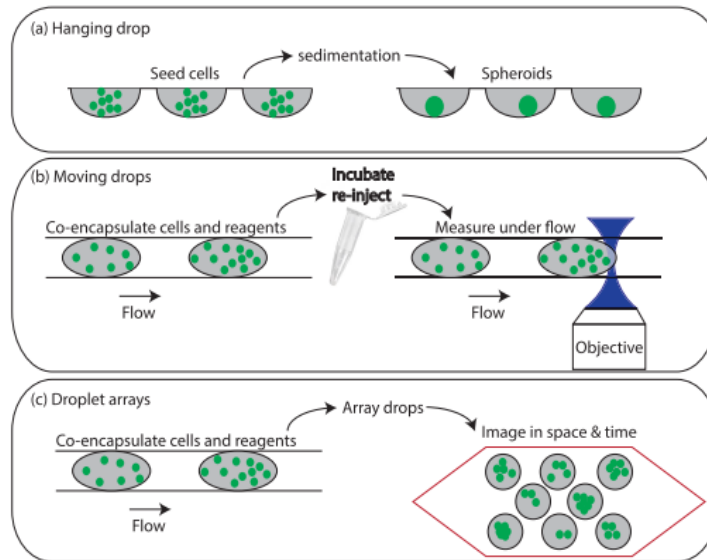


Fig. 2.11: Different types of droplet-based cell culturing platforms. The image was taken from [52].

As shown in Fig. 2.11, cell culturing methods in droplets can be divided into three types of platforms:

1. **Hanging droplets:** Droplets are suspended at the tips of microchannel outlets or in wells. This kind of platform has been used to study cell aggregation and spheroid formation [81, 82]. Hanging droplets offer a simple yet effective method for culturing cells in a three-dimensional environment, promoting the formation of multicellular structures that closely mimic in vivo tissue architecture [83]. This platform is especially useful in tissue engineering and regenerative medicine, where the development of complex, tissue-like structures is required.
2. **Moving droplet system:** Droplets are generated and transported continuously in a microchannel; at the same time, measurements are done while the droplet is on the move. By varying the flow rates and channel geometries, researchers can fine-tune the droplet size and frequency, making this platform particularly useful for applications that require large-scale screening or sequential processing of individual droplets [84]. Moving droplets can be reinjected into other microfluidic devices for further manipulation, making this a highly flexible system for complex biological assays.
3. **Droplet array:** cells are encapsulated within droplets similar to the moving droplet platforms; however, the droplets are then immobilized in an observation chamber with or without structures such as traps [85] or wells [86]. In contrast to the moving droplet system, the droplet array allows observations of the encapsulated cells over time. This platform is particularly advantageous for long-term culture and monitoring of cells, as it enables the study of dynamic processes such as cell proliferation, differentiation, and interaction with other cells or biomolecules. Droplet arrays are often used in applications where the temporal evolution of cellular behavior is important, such as in stem cell research or immunological studies.

2.5.3 General cell culture processes using droplets

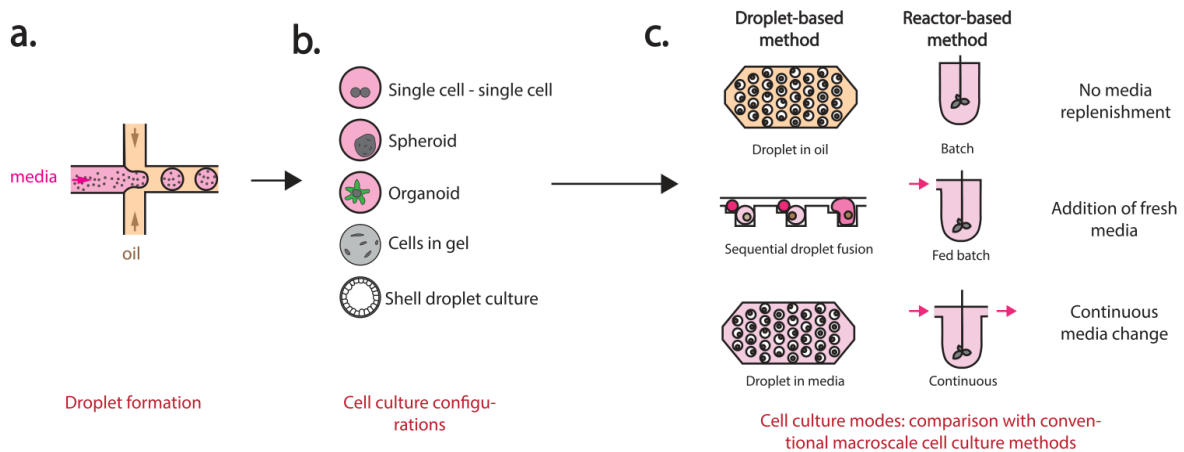


Fig. 2.12: (a) Droplet Formation: Cells are encapsulated within droplets, with the number of cells per droplet varying from one to several hundred. (b) Culture Configurations: cells can be cultured in various forms, such as single cells, doublets, spheroids, organoids, within hydrogels, or in the droplet shell. (c) Culture Modes: droplet methods can replicate different traditional culture modes. Batch cultures are represented by droplets without medium replacement, fed-batch cultures by methods like droplet pairing and fusion, and continuous cultures by replacing the surrounding oil with a culture medium. The image was taken from [52]

The process of cell culture in droplets begins with the formation of cell-containing medium droplets in oil, which is typically achieved using microfluidic junctions, as described in the

previous section. The encapsulation of cells within these droplets allows for isolated microenvironments where various cell types can be cultured individually or in small groups.

As illustrated in Fig. 2.12, there are various ways in which cells can be cultured within these droplets. Depending on the experimental goals, cells can be maintained as single entities, paired with another cell (doublets), or allowed to form more complex structures such as spheroids or organoids. A more advanced method is to incorporate hydrogels within the droplets. Hydrogels provide a scaffold that supports the formation of more advanced structures like lumen-like formations, which are critical for studying tissue-like environments and cell-matrix interactions. Additionally, it is possible to encapsulate cells within a double-layered droplet structure, often referred to as a "shell." The outer shell of the droplet typically consists of a hydrogel, which serves as a semi-permeable barrier, allowing the exchange of nutrients and waste while maintaining the structural integrity and isolation of the inner cell-containing droplet.

Cell culture methods in droplets can be compared to traditional macroscale culture modes. For instance, droplets that do not undergo medium replenishment resemble batch cultures, where nutrients are consumed, and waste products accumulate until cell viability is compromised. Alternatively, fed-batch cultures can be mimicked by periodically merging droplets containing fresh medium with those containing cells, allowing for nutrient replenishment and waste dilution. Continuous cultures, where the fresh medium is continuously supplied, can also be achieved by replacing the oil surrounding the droplets with a culture medium, facilitating ongoing nutrient exchange.

2.5.4 Poisson distribution

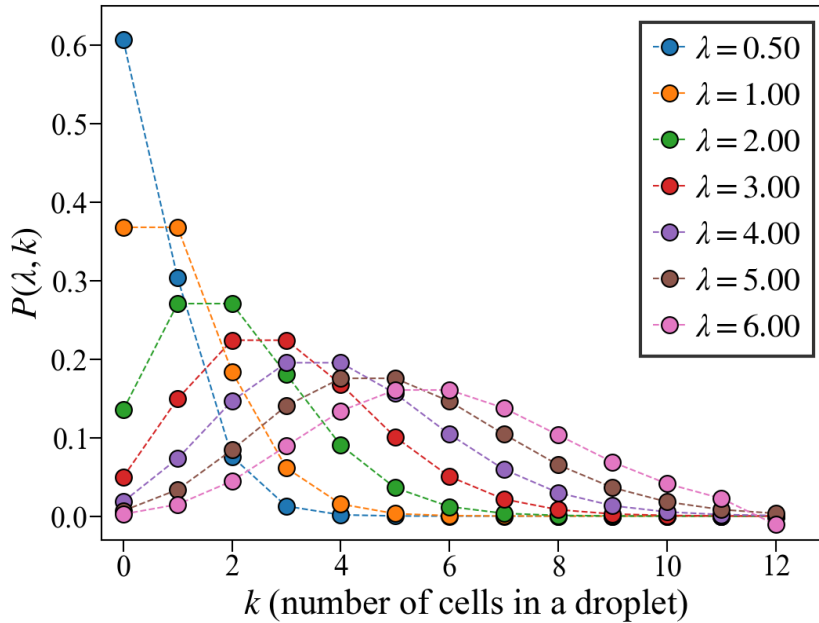


Fig. 2.13: Probability mass function of Poisson distribution for different values of the average number of cells per droplet, λ .

After cells are encapsulated in droplets, the distribution of cells within these droplets often follows a random pattern, which can be theoretically described by the Poisson distribution. This distribution is a fundamental concept in the study of cell encapsulation, particularly in microfluidic systems where droplets are generated in a consistent, controlled manner. The Poisson distribution provides a statistical framework for understanding the likelihood of different num-

bers of cells being encapsulated within each droplet, given a specific average cell concentration in the starting suspension.

In a typical droplet encapsulation process, cells are suspended in a fluid that is then segmented into discrete droplets, usually by passing through a microfluidic junction. Since the process of cell encapsulation is random—each cell has an equal probability of being captured in any given droplet—the number of cells per droplet is not uniform but follows a Poisson distribution. This means that, for a given average number of cells per droplet, λ , the probability of finding exactly k cells in a droplet is given by the Poisson formula:

$$P(\lambda, k) = \frac{\lambda^k e^{-\lambda}}{k!}. \quad (2.19)$$

Plot of Eq. 2.19 for various λ is shown in Fig. 2.13. λ can be approximated from the concentration of cells in the medium. As an example, a monodispersed droplet with a nominal volume of 0.5 nL was to be made from a suspension of 2 million cells in 1 ml of cell culture medium. The ideal average number of cells per droplet is $\lambda = 2 \text{ cells/nL} \times 0.5 \text{ nL} = 1$.

One of the key implications of this distribution is that when the average number of cells per droplet (λ) is low, many droplets will be empty, while some will contain one or more cells. For instance, if $\lambda = 1$, about 37% of the droplets will be empty, 37% will contain exactly one cell, and the remaining 26% will contain two or more cells (see Fig. 2.13). As λ increases, the distribution shifts, resulting in fewer empty droplets and a more consistent number of cells per droplet. This characteristic is particularly important when designing experiments that require single-cell encapsulation, as achieving the desired distribution often involves carefully adjusting the initial cell concentration and droplet size.

2.6 Mass transport mechanism across PDMS material.

Microfluidic chips that require a channel size of less than 100 μm are typically made from PDMS material. The fabrication of microchannels in PDMS slab involves the pouring of liquid-state PDMS into molds consisting of patterns. The patterns can be made using various techniques, such as photolithography or laser cutting, which will be discussed in the next chapter. Although the PDMS chip offers many advantages such as excellent optical transparency, gas-permeability, and accurate dimension, there is known one serious drawback: the PDMS is also liquid-permeable [87].

The mechanism is known as pervaporation[88], although in the literature, it is often called evaporation [89, 90] or water-loss [91]. The pervaporation process includes three stages: absorption of the solvent into the material, diffusion through the permeable material, and evaporation on the permeate side [88]. In materials like PDMS, it can selectively allow water to pass while retaining other solutes. The flux in pervaporation is driven by the chemical potential difference across the membrane and is a function of the membrane properties, such as permeability and thickness. This mechanism is used in applications requiring solvent removal or solute concentration without the need for external pumping devices. In the typical thick PDMS chip, the pervaporation is mainly driven by the gradient concentration of the water in the PDMS.

In droplet microfluidic, the pervaporation causes an unwanted decrease in water/medium droplet volume over time. There have been various countermeasures to prevent pervaporation in PDMS chips, such as using higher PDMS curing agent concentration, coating the PDMS with perylene [90], and using a large iso-osmotic bath [91].

Chapter 3

Material and Methods

The microfluidic chip construction consists of two or more layers of material, with at least one layer containing patterns (microchannels) and holes. The common material used for the microfluidic chip can be one or combination of the following materials:

- Solid transparent polymers such as Polymethylmethacrylate (PMMA), Polycarbonate (PC), and Cyclic Olefin Copolymer (COC),
- Glass,
- Elastomers such as Polydimethylsiloxane (PDMS).

The typical assembly methods between two layers are mechanical clamping, chemical bonding, plasma bonding, and thermal bonding. In this work, there are two main microfluidic chip fabrication techniques. The first method is based on the CNC milling, while the second method is based on the soft-lithography technique.

3.1 Chip fabrication by milling process

The first fabrication method of a microfluidic chip used in this research is based on the computer numerically controlled (CNC) milling technology. The microchannels were produced by engraving a polycarbonate (PC) plate using a milling cutter with the motion and cutting speed of the cutter controlled by a computer, as shown in Fig. 3.1. The typical microchannel width is between a few tenths to a few mm, with the minimal channel width limited to the diameter of the mill cutter.

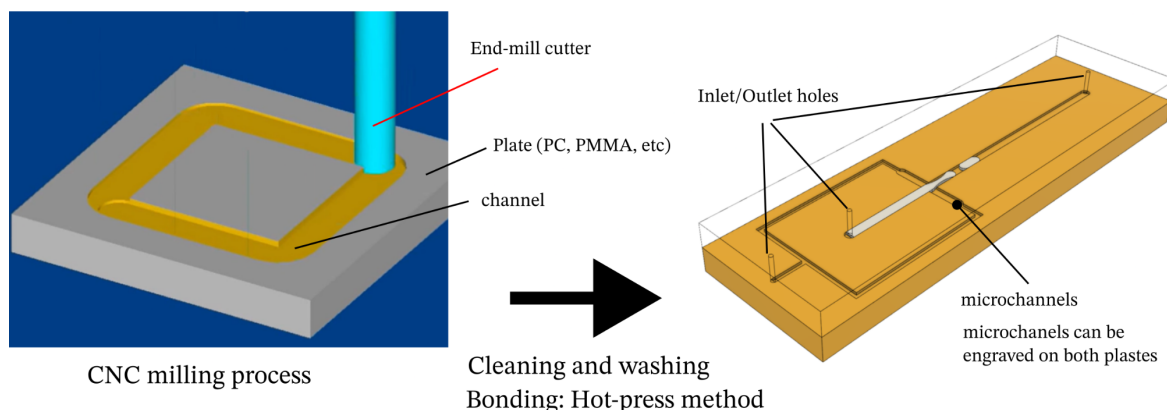


Fig. 3.1: The fabrication of microfluidic chip using CNC milling. The figure on the left illustrates the milling process to create microchannels on a plate. After machining, the plates are washed and bonded to produce the microfluidic chip

After milling, the plate undergoes a washing process using pressurized water for about 3 minutes per chip, followed by inspection under a stereoscope or microscope. The pressurized water removes the burr that remains on the edge of the microchannel. After burr removal, the chip is sequentially washed with soap liquid, distilled water, isopropanol (IPA) and again with water. Then the plates are dried in an oven at 120 °C for about 20 min before bonding.

Alignment between the two plates was done by using pins with a diameter of 1.5 mm and a length of 5 mm. Bonding of the two PC plates was done by using a hot-press machine with pressure per the area of the chip of 0.1 bar/cm² and a temperature of 135 °C for around 12 min. Then the chips were left in the hot press while it cooled in the room temperature.

3.2 Soft-lithography technique

A fundamental difference between the fabrication technique based on soft-lithography and the CNC milling is the length scale of the structure. While the minimum size of microchannels or structures in the previous technique is limited to a few tenths of mm, the photolithography technique allows the creation of structures with a size down to a few microns only. The fabrication consists of three main processes, which are illustrated in Fig. 3.2,

1. Mold fabrication using photolithography technique,
2. Pattern transfer to an elastomer, and
3. Bonding and coating of the chip.

A light-sensitive epoxy-like material, known as photoresist, is poured slowly into a substrate. The typical substrate is a 3-in Silicon (Si) wafer. Spinning of the wafer along with the already poured photoresist using a specific rotational speed generates a flat, thin film with a thickness in the order of one to hundreds of microns, depending on the photoresist viscosity and spin-coating parameter. Then, the wafer is heated to evaporate the solvent inside the photoresist, a process known as soft baking. After the soft baking, the thin film typically appears as sticky, almost-dried glue. The next step is to expose the film to UV light.

A direct write lithography machine (Heidelberg μ MLA) exposes the UV light onto a photoresist-covered wafer. The direct write technology allows the CAD to be directly exposed to the substrate without needing a photomask, thus allowing a fast prototyping process. This kind of machine generally uses a digital micromirror device (DMD) technology in that the exposure area is controlled precisely by controlling millions of tiny mirrors. In the current device, the minimum exposure area is 1 μ m by 1 μ m. Since the exposure is very precise, the inaccuracy of the resulting structure size is more affected by the exposure intensity, depth of focus, and, most

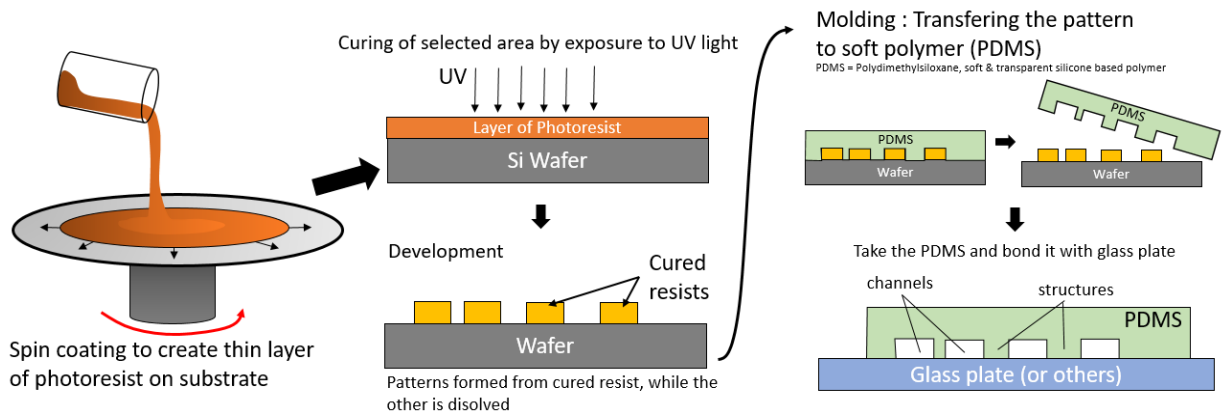


Fig. 3.2: The schematic of fabricating a chip based on lithography technique.

of all, by the spin-coating quality, which will be discussed in the next subchapter. Depending on the photoresist, the exposed thin film may need another tempering process known as Post Exposure Baking (PEB).

The next process after exposure or PEB is the development process. The most traditional method used in current research is immersing the wafer in a bath containing a developer solution. The developer is a solvent solution that dissolves uncured (non-cross-linked) photoresist and, after a duration of immersion, leaves only the cross-linked photoresist as the structure/pattern on the wafer. The area dissolved by the developer depends on the type of photoresist. In negative photoresist, the exposed area is cross-linked; thus, it stays during development. In the positive photoresist, the exposed area becomes soluble to the photoresist developer and dissolves during development. In this research, only negative photoresist is used.

At this stage, the resulting product is a wafer with cured photoresist as the pattern for the microfluidic channel, which can be called mold. This cured photoresist can be permanent or not, depending on the type of photoresist used. The non-permanent photoresists can be removed by using a solvent known as remover, with the most common available remover being acetone.

Moreover, there are at least two (optional) processes that may increase the durability of the pattern. The first method is flood exposure, in which the pattern is exposed to UV light without any masking. The other method is another heat treatment known as hard-baking, in which the pattern is heated to a temperature typically higher than PEB or soft-baking.

The next main process is the pattern transfer to an elastomer. The process is started by coating the mold with Trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (shortly as silane), i.e., a process commonly known as silanization. This process creates a very thin silane layer, which increases the hydrophobicity of the surface, making the removal of cured PDMS easier. At first, the mold is either cleaned using compressed air or rinsed using water or isopropanol (again, the cleaning fluids vary depending on the type of photoresist), followed by drying using compressed air. Secondly, the mold is put under O_2 plasma cleaner (Zepto low-pressure oxygen plasma cleaner) for 50 seconds and a power of 1W. This process will ensure that the silane attachment is evenly distributed over the surface of the mold. The second step is optional, but it can help to create an evenly coated surface for molds with deep holes, especially for those with a diameter (or nominal width) to depth ratio less than 1.

A liquid PDMS is prepared with a ratio between the silicone base and curing agent of 10 to 1. After stirring the mixture for at least 5 min, the PDMS mixture is degassed using a desiccator with a pressure of 15 kPa (0.15 Bar). After degassing, the liquid PDMS is poured slowly on top of the mold and then allowed to spread evenly. Normally, the degassing process is repeated to ensure no air is trapped inside the holes. Finally, the PDMS is allowed to cure at 60° C for at least 3 hours inside an oven.

The top layer of the chip is made from the cured PDMS, which, at this stage, is a solid elastomer. First, cured PDMS is carefully removed from the mold. The PDMS slab now has the pattern that is the cavity (“negative”) of the structure made in the mold. Like injection molding, where one mold may consist of multiple cavities, a single PDMS slab may contain multiple “cavities,” each cavity for a single chip. Upon cutting a piece of PDMS slab containing a single chip, holes for the inlet/outlet were made using a 0.75 mm Rapid-Core microfluidic puncher (Darwin microfluidic).

To ensure cleanliness, the PDMS slab is rinsed with IPA and water and dried using compressed air. Subsequently, the remaining particles or dust attached to the surface of the PDMS can be removed by attaching a piece of tape (3M Scotch Magic or Crystal tape) and then removing it. The slab is checked under the microscope, and the cleaning process can be repeated if needed.

The bottom layer of the chip is typically a 1 mm thickness microscopy glass slide. Before bonding, the glass slide is rinsed with isopropanol and water and then dried using compressed air.

PDMS slab (top) and glass slide are bonded by using O_2 plasma cleaners with the power of 1 W power for 1 minute. To increase the strength of bonding, the chip is left inside an oven

with a temperature of 120 °C for at least 1 hour. To make the microchannel hydrophobic, the channel is coated with electronic grade coating fluid (3M Novec 1720) and cured inside an oven at the temperature of 135 °C for 15 min.

3.2.1 Procedures for creating CAD for mold fabrication using maskless aligner

Although conceptualizing and sketching out ideas is a common practice across various fields, some differences exist regarding the fabrication method. Historically, photolithography technique was first developed in and later standardized for the electronic industry, only recently this technique become widely used in various fields, including microfluidic. Traditional photolithography uses a mask to select the area that will be exposed to the light. Masked photolithography is still the gold standard technique in the mass production of integrated circuits. The design consisting of single or multiple layers of mask is also known as “layout”. While in the machining process, we consider the movement of the cutting tools, in photolithography, we consider the areas that will receive exposure. Thus, there is a fundamental difference between preparing a 2D CAD for the machining process and preparing a layout file to create a mask. In CAD, the smallest unit is a point, and the movement of tools is decided by the line connecting two points. In photolithography, the smallest unit is an area with a square shape, commonly known as a “cell.” The smallest possible area of exposure cannot be smaller than a cell, and therefore a large area of exposure is a result of a collection of cells. Alas, drawing a complex shape, as in the case of a microfluidic chip, is a difficult task if one uses a cell as the building block of an area (instead of points and lines), although it is possible. Thus, the design is still drawn using 2D CAD software (AutoCAD, LibreCAD, or others), which uses points and lines (commonly known as wireframe), and later it is converted into a layout file. In 2D wireframe CAD, it is possible to draw only an area, for example, by using the hatching function. However, the resulting drawing cannot be read correctly by the μ MLA software. The μ MLA accepts various file formats, however, after hours of trial and error, it was found that the most recommended layout file format is GDSII which is a standard file format in the integrated circuit industry. Thus, the best way to prepare a layout in GDS format is by converting the CAD file (in DXF format) using a layout drawing software, for example, Klayout. To the best knowledge of the author, the following are the recommended practices for preparing a drawing for the fabrication of a microfluidic chip using a direct write lithography μ MLA.

Below is the procedure for preparing the mold design (CAD):

1. Sketch a design using any CAD software such as LibreCAD, AutoCAD, etc.
2. Set the correct unit, commonly in μm .
3. All objects in the drawing must be a closed polyline. Otherwise, it will not be recognized as an area in the Klayout. In the property of an object or a closed curve, check whether the flag is CLOSED or not. If not, use the command “join” or “pedit” in AutoCAD and use the function “create polyline” in LibreCAD for the selected closed curve.
4. If there is an area of exposure between two closed polylines, separate each polyline into a different layer in CAD. As an example, the intended exposure area is the area between two rectangles of the same center, with one rectangle smaller than the other. Then, put each rectangle into a different layer: outer layer and inner layer.
5. There are CAD objects that, by default, are not a polyline, although they look like a closed polyline, for example, a circle. The circle object is not recognized as an area either in Klayout or in μ MLA software. There is a method to convert such objects within the CAD environment; however, it normally requires multiple steps, so it is not suitable for a drawing with many circular objects. The recommended method is to use a boolean

operation in Klayout to convert many non-polyline objects into polylines in one step only, which will be discussed in the next point.

6. Save the file in DXF format only.

Next is the conversion from CAD into a GDS file using Klayout software:

1. Run Klayout in the editor mode.
2. Open the DXF file.
3. All the closed polylines are shown automatically with hatching. Non-closed polylines will not have hatching. Double-clicking on a closed object shows a “polygon” with all of its coordinates. The hatched area inside the polygon is the area of exposure.
4. Performed necessary boolean operation by going to Edit» Layer » boolean operation. For example, there are two different layers, one layer consists of inner polylines and the other layer contains outer polylines. While the intended exposure area is the area between inner and outer polylines. This can be done by using the “Difference” boolean operation. In this case, the layer containing outer polylines is subtracted by the layer consisting of inner polylines, leaving the subtracted area as the exposure area.
5. All closed nonpolylines objects (for example, circles) can be converted automatically to polygons by putting these objects in the layer containing the outer layer and performing the “Difference” Boolean operator as in the previous step. If there are no other layers, create a new empty layer and use it as the subtracting layer in step 4.

3.2.2 Important parameters and processes for creating high-quality microfluidic mold

Photolithography is a well-established manufacturing technology, particularly in the electronics industry. It provides a wide array of techniques and materials to create highly precise structures. In this research, only a few fundamental photolithography techniques have been employed to fabricate the microfluidic mold. As such, this section does not aim to cover all aspects of photolithography, which is beyond the scope and expertise of the author.

Instead, it serves as a compilation of technical notes summarizing key information and parameters essential for the fabrication of high-quality microfluidic molds. These insights can be used as a foundation for optimizing the fabrication process, which is often necessary in practical applications.

To achieve a high-quality mold, one or more process parameters must be carefully optimized. These parameters largely depend on the type of photoresist being used. For example, certain photoresists require meticulous wafer cleaning and careful handling, while others may not. The specific photoresists used in this research are listed in Table 3.1. The substrate employed in this study is a 3-inch silicon wafer. The process for fabricating a single-layer mold using photolithography is described in the following sections.

3.2.2.1 Substrate preparation (Si-wafer)

New wafers are clean, however after opening the package, dust and tiny particles may stay on the surface of the wafer overtime. These small particles may not be visible but can cause bad coating quality, such as uneven surfaces or the appearance of microbubbles on the thin film. Table 3.2 shows some essential processes and parameters for the preparation of the wafer before coating. Not all processes in Table 3.3 are mandatory. The quality of the thin film and end product determines the necessity of these procedures. For example, if the coating results are uneven or bubbles appear, consider washing and rinsing the wafer. On the other hand, if the

Table 3.1: List of resist used in current research. Thin or thick refers to the viscosity of the resist, with thick means viscous resist. Standard: the resist requires stabilization of resist at room temperature only. Moderate: the resist requires bubble removal treatment, while the wafer may or may not need special treatment. Extra: it requires all the procedure for the wafer cleaning and resist pre-treatment. Refer to section Sections 3.2.2.1 and 3.2.2.2 for recommended wafer and photoresist treatment

Resist (Manufacturer)	Type	Thin/Thick. Thickness per layer	Possibility of Multiple layers	Treatment
AZ15nXT 115cps (Micro- Chemicals)	Negative	Thin, 3–6 μm	n.a.	Standard
AZ15nXT 450cps (Micro- Chemicals)	Negative	Thin, 6–25 μm	Yes, HB is required for the preceding layer before coating of the succeeding layer.	Standard
AZ125nXT 10A (Micro- Chemicals)	Negative	Thick, 30–100 μm	Not possible	Standard
AZ1518 (Mi- croChemicals)	Positive	Thin, 1.5–3 μm	n. a.	Standard
Hare SQ-50 (Kemlab)	Negative	Thick, 20–100 μm	Yes	Extra
AR-N 4400-50 (Allresist)	Negative	Thick, 20–80 μm	Yes	Moderate
AR-N 4400-25 (Allresist)	Negative	Thick, 10–40 μm	Yes	Moderate

resulting structure can be removed too easily from the substrate, consider the plasma cleaning and the use of adhesion promoter.

3.2.2.2 Pretreatment of photoresist

This stage depends on the photoresist chosen. Thin photoresists are generally easier to handle; thus, almost no pre-treatment is needed except for keeping the temperature of the photoresist constant. On the other hand, thick photoresist tends to be more difficult to handle. It is prone to microbubbles, as air bubbles cannot escape easily from very viscous liquids. Table 3.3 shows the typical process and important parameters to ensure good quality of thin film.

3.2.2.3 Spin-coating

The spin coating consists of at least two main spins: the spreading photoresist and the set film thickness speed. However, it can be challenging to obtain good quality and repeatable coating. Below are some important procedures and parameters to achieve good quality of film:

- **Method of pouring** (critical parameter). Direct pouring from the small vial is recommended. The use of a pipette or syringe is not suitable for thick photoresists. Pour photoresist carefully, such that there is no air bubble trapped. A thin stream of thick photoresist during pouring tends to trap bubbles. Pour such that at least 75% of the substrate is covered by the photoresist. The coverage of substrate by photoresist is important to allow repeatable film thickness, especially in thick photoresist. Pour photoresist in the center of the wafer: allows better uniformity of the surface.

Table 3.2: Recommended processes for cleaning the Si-wafer

Process	Recommendation
Washing and rinsing	Cleanse the wafer from dust or unwanted particles. The recommended process is: 1) Ultrasonic bath for 15 min in acetone, 2) rinsing with IPA, and 3) drying with air.
Dehydration	Drying of the wafer after washing and rinsing. Typical process: 150°C for 3–10 min on a hot-plate. Increase time twice or thrice in oven drying.
O ₂ Plasma Cleaning	Clean the wafer and increases the wetting-ability of the surface. Thin film is more even and has smaller edge bead. Typical parameter: 100% power for 15 min. Activated wafer attracts dust. Time between plasma cleaning and coating should be as short as possible.
The use of adhesion promoter	At first, wash and rinse as mentioned above, followed by dehydration. Second, apply adhesion promoter and spin it until dry (spin speed follows manufacturer recommendation). Adhesion promoter is volatile, after it dries out, immediately pour resist and spin.

- **Rotational speed for spreading the photoresist** Apply low rotational speed spin to spread the photoresist evenly over the wafer. The spin should be started as soon as possible after pouring. Typical parameters: 300–400 RPM for 10–30 s.
- **Snap edge spin.** Apply high rotational speed spin for 1 s or less. It helps to let the photoresist reach the edge and remove the extra photoresist on the edge of the wafer. The speed can be higher than the set-film speed. This step is required only for thick photoresist coating.
- **Set film-thickness speed and time.** Apply the rotational speed that will determine the thickness of the thin film. The film thickness is determined by the combination of two parameters: rotational speed and time. The centrifugal force— mS_{ang}^2r where m , S_{ang}^2 , and r are mass, rotational speed, and radius, respectively—increases linearly with the radius and is balanced by the viscosity of the photoresist. Given enough time, these two forces balance, resulting in a uniformly thin film. The setting of rotational speed and time depends on the viscosity of the photoresist:
 - Thin photoresist: low viscosity photoresist flows easily. Thus the balance of force is reached after a short spinning time. For typical spinning time (>20 s), the film is stable, additional spinning time will not change the thickness, unless the speed is increased. Thus, for thin film coating, to get the desired thickness, one needs to adjust the speed only while setting the spinning time long enough (typically 30–60 s).
 - Thick photoresist: viscous photoresist flows slowly, so the balancing of forces takes a longer time. Due to this uncertainty, to get the desired film thickness of a thick photoresist, it is easier to adjust the spinning time while setting the speed to be constant (typical speed is >1000 RPM). Because of the same reason, the amount of poured photoresist becomes important for thick photoresist. Higher amount of poured photoresist resulting in a thicker film. Thus, to get repeatable film thickness, one must be able to control the amount of photoresist poured. One easy way is to transfer the photoresist into a small vial, as described previously.

Table 3.3: Photoresist preparation before coating

Process or parameter	Recommendation
Temperature of resist	Critical parameter. Resist must be at room temperature before opening of the resist container. Low temperature of resist cause condensation of water vapor in the air; thus, it increases the water content of the resist and may create problems such as bubbles. Typically, the resist is left in the room overnight. Ensure the resist is at room temperature before opening the bottle to prevent condensation of water that may change the properties of the resist.
Transfer to a smaller container	Pour resist into smaller glass bottle, typically 10 ml glass vial. This procedure is recommended because it allows for: Prevention of contamination of resist in the original bottle. Resist pouring directly inside the spin-coater, permitting immediate spin. Better control of pouring (prevent bubbles) and control of the dispensed volume.
Pre-heating	50°C overnight in an oven. Mostly only for thick SU-8 and Hare-SQ resists. It reduces the resist viscosity to allow release of microbubbles.
Ultrasonic bath (resist)	10-15 min ultrasonic bath for bubble removal. Do not sonicate too long that the bath becomes too hot. Repeat if needed, but keep the temperature of the bath low.

- **Acceleration** Set the rotational speed ramping according to the photoresist manufacturer recommendation. Typical acceleration is 200–1000 RPM/s.
- **Edge bead removal.** Removing the edge bead by using special solvents or developer solutions. The wafer is spun at a speed lower than the set film thickness speed (typically 500–1000 RPM). The solvent is injected onto the edge of the wafer, through a syringe and needle, with a typical flow rate of 100–200 ml/h. The solvent dissolves the film; the spinning ensures the solvent flows in the outward-radial direction.

3.2.2.4 Soft-baking

The photoresist is heated to evaporate its solvent, and by the end of heating, the photoresist becomes more like a solid substance. At the early stage of baking, the photoresist softens and may flow. The receding photoresist flow from the wafer edge to the center increases the edge bead size and causes a non-uniform surface thickness, which is unavoidable unless edge bead removal is performed beforehand. At this stage, the microbubbles are released. If the film contains too many bubbles, the uniformity of the film is destroyed, and the surface contains crater-like holes. To avoid such a problem, the photoresist and wafer pre-treatment procedures described previously are necessary. Below are some important products and its parameter for good practice of soft-baking:

- **Soft bake temperature and time.** The recommended temperature and duration are provided from the photoresist datasheet. Not enough soft-bake causes easy removal of the cured photoresist during development, whereas too much soft-bake may cause difficulties during the removal of the uncured photoresist during development. The typical procedure in this research uses two-stage heating: 1. Pre-heating at a lower temperature and with shorter time and 2. Soft baking. Use temperature ramping if needed. Pre-heating is not

critical, but it helps to reduce thermal stress, and it is a faster method compared to using ramping temperature.

- **Uniformity of heating (hot-plate).** The uniformity of heating is important when soft-baking is done on a hot plate and done for a large wafer or structure. Cleaning the hot plate surface before soft bake to ensure the smooth surface of the hot plate. If the hot plate surface is not flat enough, use a glass plate on top of the hot plate to allow uniform heating.
- **cooling.** After the soft-baking, allow slow cooling of the water to room temperature by putting the wafer on a piece of glass, for example, on a glass petri dish.

3.2.2.5 Exposure

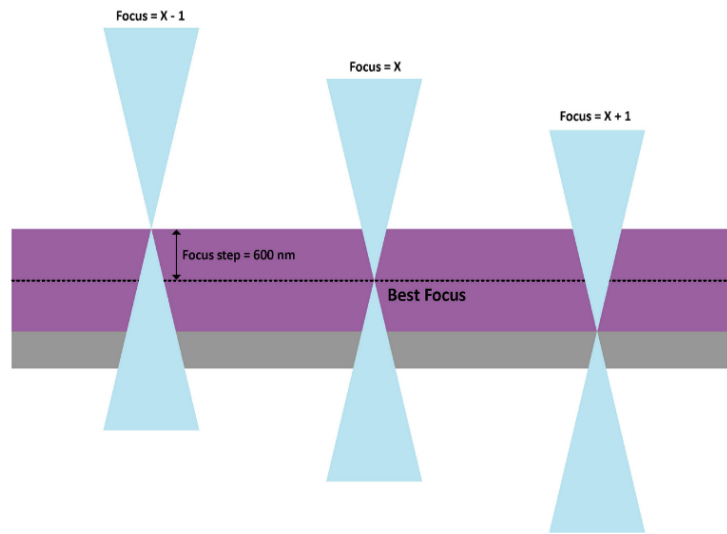


Fig. 3.3: Illustration of the UV beam focus position on the substrate. The image was taken from [92]

This section discusses various factors that are important for the exposure process using mask-less aligner Heidelberg μ MLA (Heidelberg Instrument). Two parameters used in the exposure using μ MLA are Dose and Defoc. Assuming an exposure of a negative photoresist film on the Si-wafer, below are some discussions about those parameters and additional precaution steps:

- **Dose [mJ/cm^2].** The UV light dose plays a critical role in defining the quality of the resulting structure. Insufficient exposure can leave portions of the photoresist uncured, causing them to dissolve during the development process. Signs of underexposure include structures with smaller dimensions and rounded or blunt edges. In contrast, overexposure typically leads to structures with larger dimensions than intended. Additionally, areas meant to remain uncured, such as holes, may become fully exposed and develop as solid features. A photoresist is typically sensitive to a specific UV wavelength. Four commonly used UV wavelengths in photolithography are the g-line (436 nm), h-line (405 nm), i-line (365 nm), and broadband (280–315 nm). For example, the AZ series photoresists (Microchemicals, Germany) are designed for sensitivity to the i-line, while the μ MLA machine employed in this research operates at 390 nm UV. There is no clear guideline for determining the required dose when the exposure wavelength falls outside the specified range of the photoresist. In such situations, it is advisable to consult the photoresist manufacturer for guidance. If manufacturer support is unavailable, adjustments must be determined experimentally through trial and error. For the AZ series photoresist,

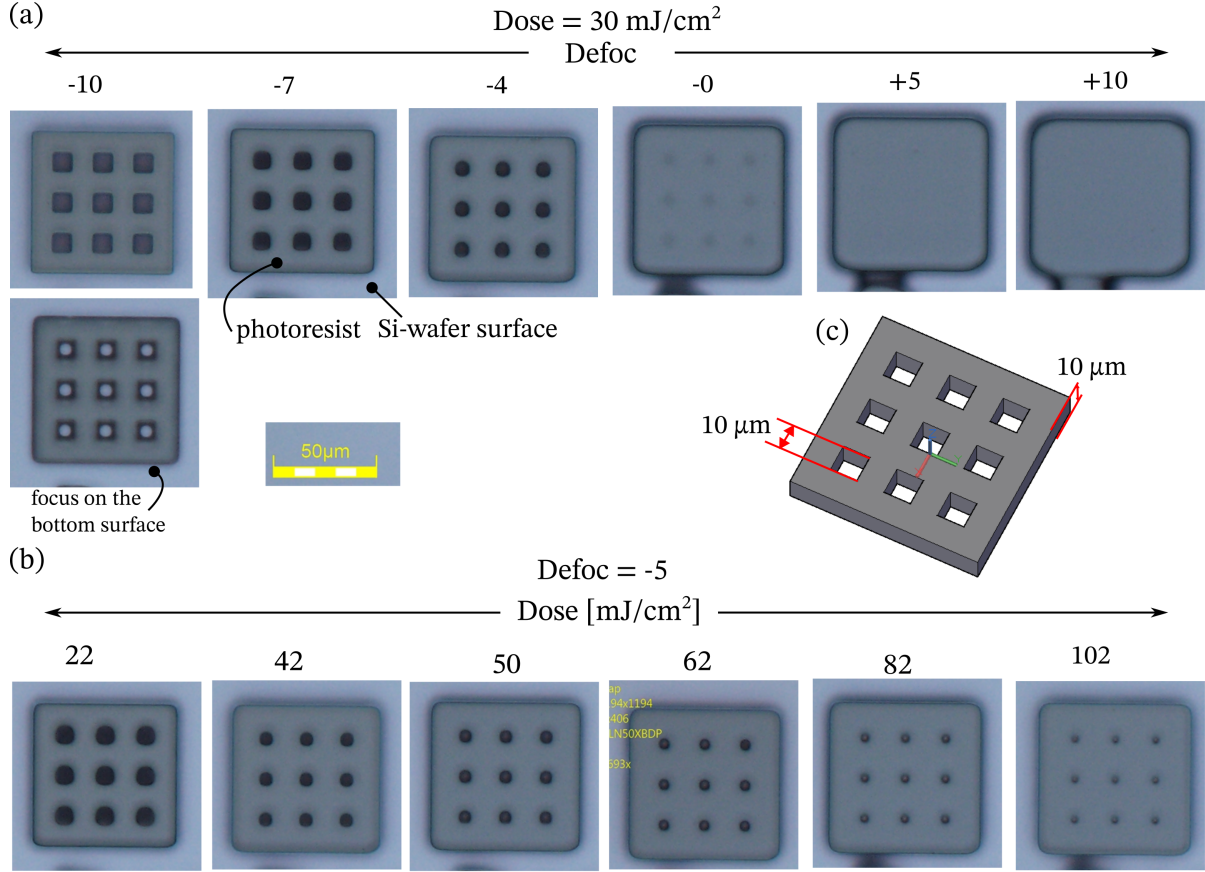


Fig. 3.4: Images of the cured photoresist viewed from the top. (a) The effect of parameter Defoc with constant exposure dose. (b) The effect of exposure dose (Dose) of the UV-light. (c) 3D illustration of the test structure. The structures have 10 μm thickness. The photoresist used is AR-N 4400-25 (AllResist).

exposures at non-ideal wavelengths generally require a higher dose, ranging from 150% to 200% of the recommended dose. Some photoresists may fail to cure entirely if exposed to wavelengths beyond their specified sensitivity range. The comparison of the cured photoresist exposed using various Dose values is shown in Fig. 3.4b.

- **Defoc**[-10 to 10, each step is a shift of about 600 nm]. Fig. 3.3 shows the schematic of the UV beam passing through the photoresist film. Borrowing the term *draft angle* from injection molding technology, the angle of the side face of the cured structure can be adjusted by moving the focus position either lower or higher. The ideal focus should be located in the middle of the thin film, and the resulting structure should have a zero draft angle, i.e., a straight structure. Shifting the focus lower (positive increment) or higher (negative increment) results in a structure with positive and negative draft angles, respectively. A negative draft angle means the structure is narrower at the base and wider at the top. This condition should be avoided because it will cause difficulty during PDMS removal in the later stage of fabrication. A negative increment is useful when fabricating structures with high aspect ratio holes (i.e., the hole is deep but narrow). With zero or positive increment Defoc, the lower or whole part of the hole is filled with cured photoresist. Negative increment Defoc reduces the exposure intensity at the lower region of the photoresist, preventing the overcuring of the photoresist surrounding the hole, thus allowing the creation of high aspect ratio holes. In this research, it was possible to make holes with a height-to-width ratio of approximately 3. The effect of various Defoc values exposed with constant Dose is shown in Fig. 3.4a.

- **Cleaning the back of the wafer.** After spin-coating and soft-baking, there can be a photoresist left attached to the back of the wafer. This photoresist can be wiped off using a napkin soaked with Acetone (or other remover). Also, clean the back of the wafer if there are particles. Particles and photoresists attached to the back of the wafer cause a non-horizontal wafer on the μ MLA machine.

3.2.2.6 Post Exposure Baking (PEB)

Post-exposure baking (PEB) is another baking step that is performed right after the exposure. Some photoresists do not require PEB, as the cross-linking is done during the exposure, for example, the AZ 125 (Microchemical). The other photoresist requires PEB to achieve the cross-linking of the exposed photoresist. The majority of photoresists used in this research required PEB. The temperature of PEB is typically higher than the temperature of soft-baking. A typical sign of a good PEB is that the exposed structure becomes visible after baking. The first layer (or the previous layers) of a multilayer structure should have PEB at a temperature and duration much lower than the typical single-layer structure to avoid over-baking, which could lead to issues during development.

3.2.2.7 Development

The wafer is immersed in a developer solution, which dissolves the non-cross-linked region, revealing the structure. There are a few important factors affecting this stage:

- **Development time.** Leaving the wafer in a developer bath for too long may dissolve all the thin film, including the exposed region. Over-baking—either the baking temperature or duration is too high—increases the time for development.
- **Rate of liquid stirring.** Stirring the liquid by shaking the container accelerates the dissolving process, although it may not be necessary.
- **Rinsing liquid.** Use the liquid recommended by the manufacturer. Typical rinsing liquid is either distilled water or IPA.

3.2.2.8 Hard-baking (HB) and Flood Exposure

Both HB and flood exposure are optional processes. Hard-baking is a thermal process that hardens the structure by increasing cross-linking and removing unwanted solvents. It may also reduce the formation of cracks. HB is typically performed at higher temperatures than other thermal processes. However, if the temperature is too high, it can cause “re-flow,” where the cured photoresist begins to revert to a liquid phase. HB in an oven generally requires more time than HB on a hot plate. To ensure a uniform heating process, it is recommended to perform HB in an oven rather than on a hot plate. Another method for making the structure permanent involves exposing the mold to UV light for a specified duration. The UV light enhances the cross-linking of the photoresist, with the exposure time depending on the required intensity (from the photoresist) and the UV lamp’s power density. For example, if the photoresist requires 10,000 mJ/cm² and the UV lamp provides 200 mW/cm², the necessary exposure time would be calculated as:

$$\frac{10,000 \text{ mJ}}{200 \text{ mW}} = 60\text{s}.$$

3.2.3 Multiple layer coating

For a multiple-layer structure, a multiple-coating process is required, which results in a more challenging process than a single-layer fabrication. A common fabrication step for a single layer mold is given as 1. Spin-coating → 2. Soft-baking → 3. Exposure → 4. PEB → 5. Development

→ 6. Optional steps such as hard baking or flood exposure. In two or more layer fabrication, there are two ways to accomplish this:

1. Performed first layer fabrication as usual (e.g., steps 1—5 or until step 6) and then performed steps 1–5 for the next layer and so on.
2. Performed steps 1–4 for the first layer, then continued with steps 1–4 for each subsequent layer. After all the layers have been coated, perform the development process (step 5) and the following steps, such as hard-baking or flood exposure [93].

Method 1 takes more time because of the repeated development steps (step 5). An additional problem comes from a thick photoresist. The viscous photoresist cannot fill the holes with the first layer structure during spin-coating, thus creating holes. The resulting film is also uneven due to the coating on an uneven surface. Depending on the viscosity of the photoresist, the rotational speed, and the thickness of the preceding structure, the subsequent coating may follow the contour of the first layer structure, creating an uneven film. Mostly, it is not the case for thin photoresists due to low viscosity and if the first layer structure is smaller (thin) enough compared to the thickness of the subsequent layer. The main advantage of method 1 is better thermal management. The unexposed region of the preceding layer will not receive any thermal treatment of the subsequent layer process. Method 1 is also very useful for the initial trial, in which inspection of each layer can be performed before starting the subsequent layer process. Any additional step can be executed before coating the subsequent layer. For example, as listed in Table 3.1, the preceding layer in multiple-layer coating using AZ15 450cps requires hard-bake before the coating of the subsequent layer.

Fig. 3.5.

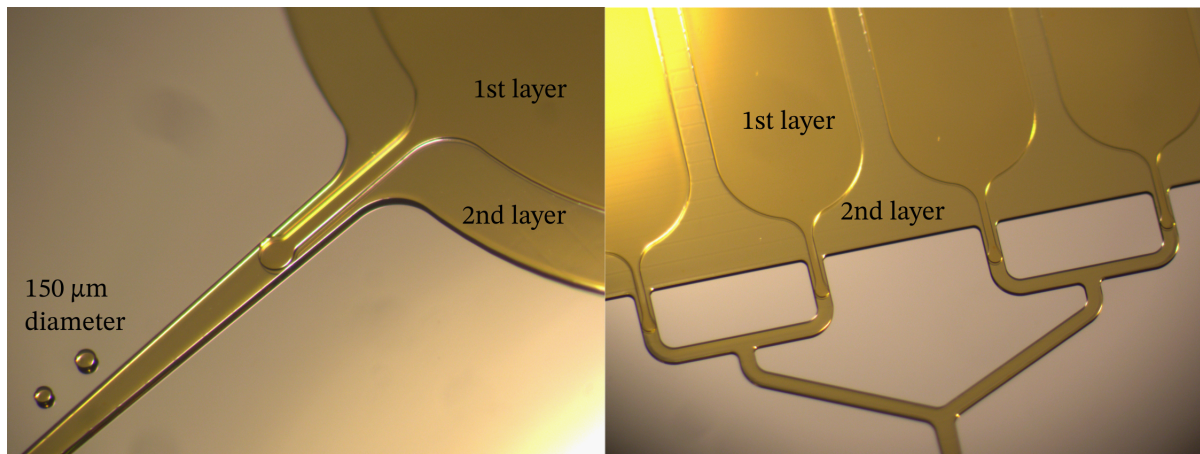


Fig. 3.5: An example of two-layer fabrication result. The first and the second layer thickness are $60\ \mu\text{m}$ and $24\ \mu\text{m}$, respectively. The photoresist used is the AR-N 4400-50 (AllResist).

Method 2 is clearly ahead of method 1 regarding overall fabrication time. It is also a favorable method for the thick photoresist (and the relatively thick structure of the preceding layer) because the subsequent layer coats on the flat preceding layer. Thermal management is the challenge in method 2. The unexposed region must undergo a series of baking steps on the subsequent layer. Due to excessive baking, the unexposed region may accidentally be cured, so removing it during the development process is impossible. Method 2 requires initial trial and error to get a good recipe. As a rule of thumb, The temperature and the time for soft-bake and PEB of the preceding layer should be reduced. An example of two-layer fabrication results using method 2 is shown in

3.3 Experiment setup for the study of droplet formation

For the study of droplet formation, six cross-junction devices were prepared, as shown in Table 3.4. Five of them are devices with a rectangular cross-section, each with a different aspect ratio, and the other one with a circular cross-section. The experiment setup is shown in Fig. 3.6. Working fluids were delivered using separate computer-controlled syringe pumps (Cetoni Nemesys) loaded with 1ml glass syringes (ILS) or 500 μ l glass syringes (Hamilton Luer-Lock series). Initially, testing was performed at the lowest flow rates to ensure that no flow fluctuations could be caused by the mechanical components of the pump in the range of parameters investigated in this study [4]. The syringes were connected to the inlets of the devices using Polyethylene (PE) tubing (Beckton-Dickinson PE-60) or Polytetrafluoroethylene (PTFE) tubing with an internal diameter of 0.80 mm (Bola). The same tubing was used to connect the outlet of the devices to a waste container. In this study, the flow rate range is 0.01 ml/h to 14 ml/h, covering a range of Ca between 7×10^{-6} and 5×10^{-3} .

The CP and DP liquids are hexadecane 95% (Sigma Aldrich Co.) and fluorinated oil FC-40 (3M), respectively. The fluid pairs were chosen to ensure the wetting condition of the PC surface channel with CP without the need for surfactant. Thus, the effect of surfactant is excluded. The interfacial tension between CP and DP, γ , is 7.3 mN/m, measured using the pendant drop method at 21 °C. The viscosities of hexadecane and FC-40 are $\mu_C=3.6$ mPas and $\mu_D=4.1$ mPas, respectively, measured using a falling ball viscometer at 21 °C.

Table 3.4: The dimension for the six chips prepared for the study of the droplet formation.

Microchannel cross-section	Width [μ m]	Height [μ m]	Aspect Ratio W/H [-]
Rectangular	2010	494	4.07
Rectangular	1510	495	3.05
Rectangular	955	469	2.04
Rectangular	752	525	1.43
Rectangular	501	525	0.95
Circular	Diameter: 400 μ m		

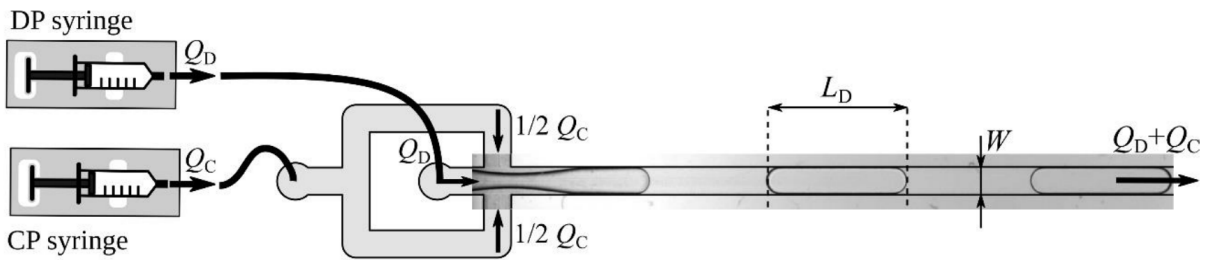


Fig. 3.6: The schematic of the experiment setup for droplet formation in cross-junction device.

3.4 Experiment setup for the study of droplet incubator

3.4.1 Initial set up and cells supply problem

The initial setup looked very similar to the setup used for the study of the droplet formation, except that the size of the microfluidic channel is much smaller (see Fig. 3.7). The height of the channel by design is 60 μ m, while in actuality, the thickness is in the range of 40–80 μ m depending on fabrication. Two glass syringes (Hamilton 1700 series), each with a capacity of either 100 μ l, 250 μ l, or 500 μ l, were used to supply the CP and DP liquid into the cross-junction. CP is

a fluorinated oil (3M FC-40), whereas DP is a medium with cells. The cross-junction produced much smaller droplets, having a volume range of one-tenth to one nl. Droplets containing cells are then collected in the incubator region, where observation of the cells' activity is performed.

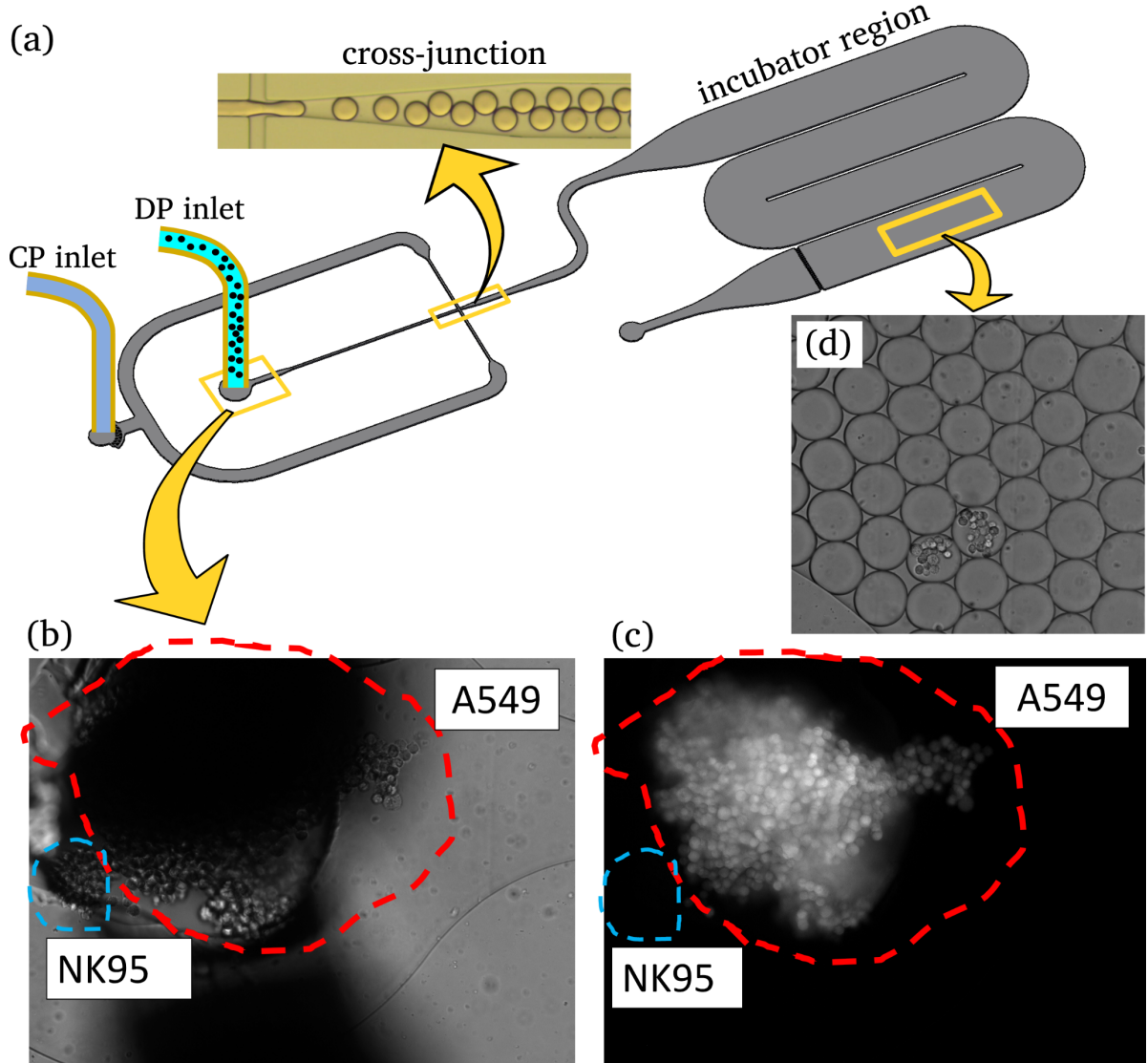


Fig. 3.7: (a) Schematic of the initial experiment setup. Agglomerates of (b) NK cells (NK95) and (c) the cancer cells (A549) at the DP inlet. (b) and (c) are bright field images and fluorescence microscopy images, respectively. (d) No cells encapsulated inside the droplets, or there is a clamping of cells within one droplet.

Different from the previous experiment, the DP now contains a medium with cells; thus, it requires a different experimental procedure. Before the experiment started, the DP syringe and the tubing were filled with either medium (without cells) or fluorinated oil. Air is removed from the syringe and tubing, and some amount of fluid is dispensed from the end of the system. Then, the end of the tubing with its connector is inserted into a vial containing a medium with cells. A backward plunger motion (refill syringe) fills the tubing with the cells-containing medium. Subsequently, the tubing is inserted into the DP inlet, as depicted in Fig. 3.7a.

Initial experiment results have shown that this procedure or method does not allow the cells to enter the chip correctly. The cells agglomerate inside the tubing, and they enter the chip as clumps of cells, as shown in Figs. 3.7b and c. Those clamped cells still went into the cross-junction and encapsulated in a droplet, however, the resulting droplets contain no or rarely with too many cells, as shown in Fig. 3.7d. There are three reasons that contribute to this problem:

1. The distance traveled by cells inside the tubing until it reaches the cross-junction is too long. Cells containing medium approx. fill at least the last 100 mm of the DP tubing (ID 0.80 mm). Ideally, cells are distributed along this length. However, it may happen that the distribution is not homogeneous; for example, a higher concentration of cells may accumulate upstream of the tubing as a result of non-ideal mixing.
2. The flow rate inside the tubing upstream of the chip inlet is too low. The average DP forward speed is between 5 and 22 $\mu\text{m/s}$, calculated using tubing with ID 0.80 mm. For consideration, the sedimenting velocity of a cell can be approximated using Stoke's Law as much as 1 $\mu\text{m/s}$. It seems negligible, but it is comparable to the forward speed of the medium, which means that the longer the cells stay in the tubing, the more possibility they clamp to each other.
3. The time taken from cell loading to the tubing until the start of the experiment may be too long.

The combination of these three reasons causes cells to clamp together inside the tubing, provided by limited space inside the tubing. Changing the tubing's internal diameter did not help. Using smaller inner diameter tubing provides higher entry speed but with a longer distance to travel since the same volume of medium occupies a longer length of tubing. Using larger inner diameter tubing only resulting the opposite effect, but it does not solve the problem. Cells need to be well mixed and to be encapsulated as soon as possible, which means the travel distance and time of the cells must be very short.

3.4.2 The use of pipette-tip as DP inlet and the negative pressure device

The issue was at first solved by using a pipette tip as the end of the DP tubing [94, 95], with the driving pressure as shown in see Fig. 3.8a. A PDMS plug with a thickness of 3.5 mm was prepared with a hole in its center for the connection to the tubing (Bola PTFE tubing 0.8 mm) and a connector (Darwin Microfluidics 18G Stainless Steel 90° Bent PDMS coupler). As an alternative, a trimmed stainless steel luer lock needle (Unimed, 0.8×35 mm) can be used instead of a pipette tip, as shown in Figure 2.5a. The advantage of using a trimmed steel needle is the smaller size compared to a pipette tip, allowing the chip to be operated on an inverted microscope. An inverted microscope has a condenser lens located not very far from the table, resulting in a limited space between the chip and the condenser lens. The procedure is slightly different from the previous setup, the DP syringe and tubing assembly (including the pipette tip or trimmed steel needle) is filled with either medium or fluorinated oil until air is removed completely from this system. Afterward, some oil/medium is pushed out from the system to allow the filling of the pipette tip with cells containing medium. Then, the pipette tip is inserted into the inlet of the chip (see Fig. 3.8a), and the experiment is started.

This method allows the localization of cells in an area inside or very close to the chip. Moreover, the pipette tip (or steel needle if long enough) can be inserted until the tip enters very close to the microfluidic junction. Thus, it significantly shortens the distance traveled by cells, preventing them from clamping together. The use of a pipette tip is better than the use of a steel needle. A large portion of cells containing medium is inside the luer-lock space of the needle. Accumulation of cells on the bottom of the luer-lock space was observed if the filling and subsequent procedure took too much time. If the space from the top of the chip to the microscope component is not an issue, then the pipette tip is the better option. Overall, if the procedure was done smoothly and as quickly as possible, there would be no clamping of cells, and the resulting droplets contain a single or few cells only, as shown in Fig. 3.8c.

In practice, the use of pipette-tip or steel needle assembly to some degree is troublesome. First, the filling and insertion process may take some time, which affects the distribution of the cells and the resulting number of cells in each droplet. Second, the insertion of the pipette

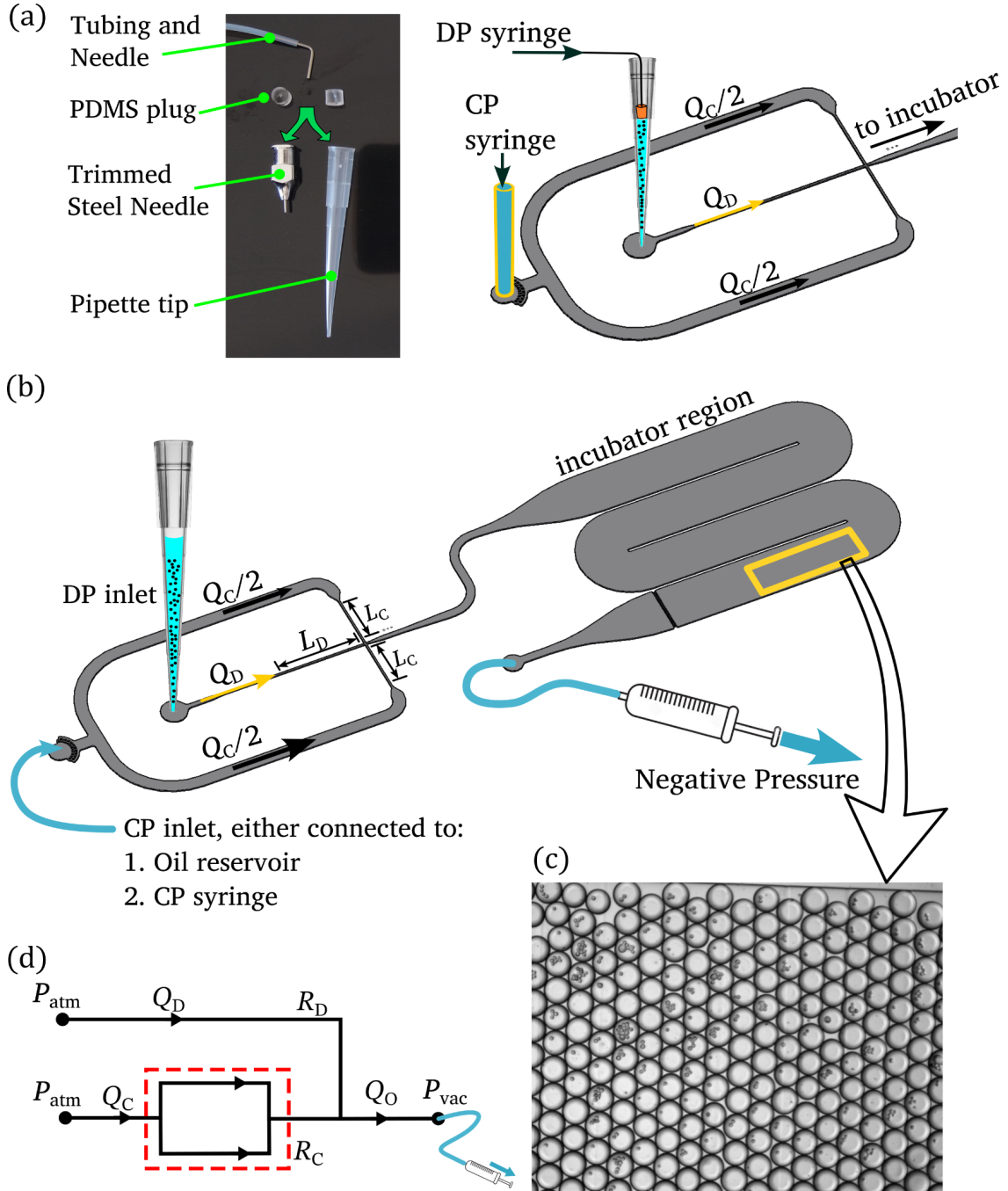


Fig. 3.8: (a) The schematic of a pipette tip or steel needle assembly for cells and the modified experiment setup using positive pressure supply (b) The negative pressure-driven system. Only one syringe is required. The CP inlet can be connected to the oil reservoir or to a syringe. (c) The experiment results show the majority of droplets contain only a few cells. (d) Flow circuit model of the Negative Pressure Device inlets.

tip/steel needle is prone to air and contaminating agents, resulting in bubbles and/or contamination that has proven to be very difficult to remove from the system once it is inside the chip. The previous reports [94, 95] use the same principle but with two separated chips, i.e. the droplet generator chip and the droplet incubator chip, allowing easier bubble and contamination removal.

The latter issue was solved using negative pressure device system (NPD) [96, 97]. The basic NPD configuration is shown in Fig. 3.8b. In the DP inlet, there is only a pipette tip (10–300 μ l

size) filled with the cells containing medium. The CP inlet can be connected to a large well of oil reservoir made on top of the chip or to the oil reservoir by tubing. The outlet of the chip is connected to a syringe (Hamilton 1700 series 250 μl) filled with fluorinated oil. Only one syringe is required in this system, and this syringe provides a negative pressure, creating a flow existing in the chip, Q_O , as shown in Figs. 3.8b and c).

Removal of air bubbles and cells loading are effortless in this setup. Positive flow of oil (into the chip) Q_O to flush out the air through the CP and DP inlet holes. Normally, there is a large well of oil on top of the chip, created by bonding a wall shape PDMS on top of the chip. The exiting oil due to flushing is collected in the top well while the air bubble is gone. Loading of cells is performed using a pipette. At first, the cells containing medium were mixed by sucking and dispensing the medium repeatedly by pipette. Then the medium is loaded into the pipette with a volume around 100 μl . The minimum volume to produce around 10^3 droplets is less than 10 μl . However, the extra medium is available as cells were prepared in a larger volume of fluid, and this extra volume is useful to allow longer experiments such that it can avoid an empty pipette before the experiment ends. Then, the pipette tip is injected into and left attached to the DP inlet while the pipette is removed. The experiment is started by providing negative flow Q_O (flow exiting the chip). The negative pressure sucks the DP and CP fluids into the cross-junction, and the droplet is produced. The straightforward cell loading and bubble removal procedures allow more repeatable experiment results. The result shown in Fig. 3.8c becomes the norm, i.e., there is no clamping of cells, and bubble entrapment rarely occurs. The loading procedure no longer affects the cell distribution inside the medium as long as the pre-mixing is done properly. Thus, the number of cells inside the droplets became strongly correlated to the cell concentration in the medium.

Another advantage of using an NPD device is that it allows the flow rate ratio ($q = Q_D/Q_C$) between the DP and CP to be embedded on the chip design, eliminating the need of CP syringe. With constant and common cross-section area of the channel before the cross-junction (see Fig. 3.8d), q can be set by choosing the correct length ratio between them:

$$q = \frac{R_C}{R_D} \approx \frac{\mu_C L_C}{2\mu_D L_D}.$$

R_C and L_C are the hydraulic resistance and length of the CP inlet channel, respectively, while R_D and L_D are those of the DP (see Figs. 3.8b and d). The viscosities of the CP and DP are μ_C and μ_D , respectively. Alternatively, a CP syringe can still be used to provide a positive flow of CP, and the above length ratio can be neglected. In this case, q is defined as:

$$q = \frac{Q_O}{Q_C} - 1.$$

As long as $Q_O > Q_C$, there will be droplet production.

3.4.3 Fabrication modification to prevent droplet pervaporation

To reduce the mass transport of the droplet liquid through the PDMS, three different constructions of microfluidic chip were tested. The first type is the PDMS-glass chip with an additional liquid well on top of the chip, as shown in Fig. 3.9a. The liquid pool was made by cutting a section PDMS slab and then bonding the section into the PDMS by using the plasma bonding (see Fig. 3.9b).

The second type is the PDMS-glass chip with non-water-permable material insert (see Fig. 3.10). The insert can be transparent polymer or glass. In this study, two kinds of insert were used: PET film (200–400 μm thickness) and microscopy coverslip (150 μm thickness). The insert was placed right after pouring the PDMS liquid into the mold. The cover slip was carefully placed on top of the incubator region pattern (photoresist) using a pipette tip. No additional modifications are needed in the fabrication process, except that the PDMS slab should be released from

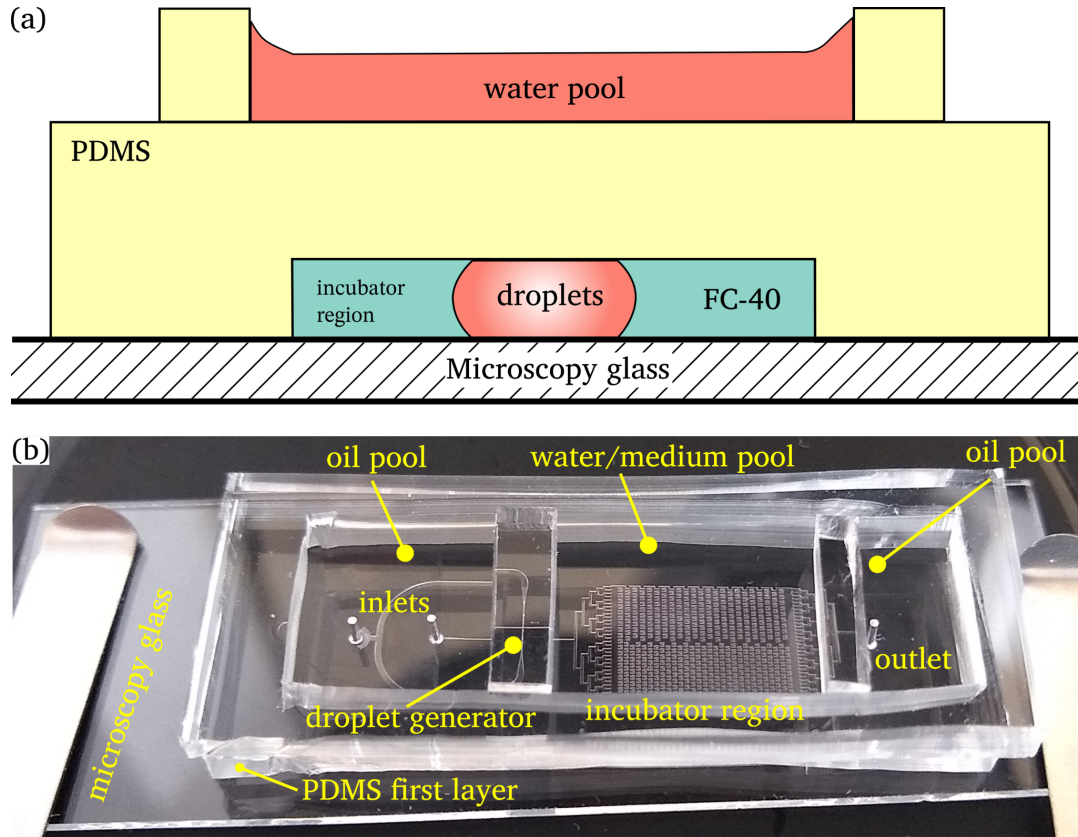


Fig. 3.9: (a) Schematic of the cross-section of the incubator region and (b) the actual image of PDMS-glass chip with water pool on top of the chip. The water/medium pool is used to reduce the pervaporation effect of the droplet liquid through PDMS material, and it is located above the incubator region of the chip. The oil pools above the inlet and outlet ports are essential for preventing air from getting trapped when connecting or disconnecting the tubing connector or pipette tip. Additionally, they help to keep the chip from drying out due to oil evaporation.

the mold very slowly to prevent damage to the coverslip and photoresist. With this method, the insert is implanted inside the chip as shown in Fig. 3.10a, and there is always a very thin layer of PDMS below the cover slip. The thin PDMS layer protects the glass from breaking and also allows for gas exchange. Additionally, the chip also uses a side channel (see red in Fig. 3.10b), that is, to saturate the PDMS with water from the sides.

The third type of chip does not use the PDMS material at all: glass-glass chip. The glass top plate, which has a thickness of 1 mm and consists of microstructure, was made by glass etching process (SINTEF, Norway). There are two types of construction in the glass-glass chip:

1. **PSA Chip.** The top glass plate, which contains microstructures, is bonded to a standard 1 mm microscopy glass slide using a pressure-sensitive adhesive (PSA, Adhesive Research 90445). Figs. 3.11a and b show a schematic of the glass-glass chip and the actual assembled chip.
2. **Constructible Chip.** The components of the constructible chip are illustrated in Fig. 3.11c. Both the top and bottom plates were made from 4 mm polycarbonate (PC) and were prepared using a CNC milling machine. The top plate includes inlet and outlet ports, while the bottom plate features an O-ring groove. A 1 mm thick glass plate with microstructures is positioned between the bottom and top plates, with an O-ring (18 mm internal diameter and 0.5 mm cross-section diameter, Polymax) compressed between the bottom plate and the glass plate to provide a seal. To minimize the deflection of the top and bottom plates, a spacer (middle plate) made from 1 mm thick PC is placed between them.

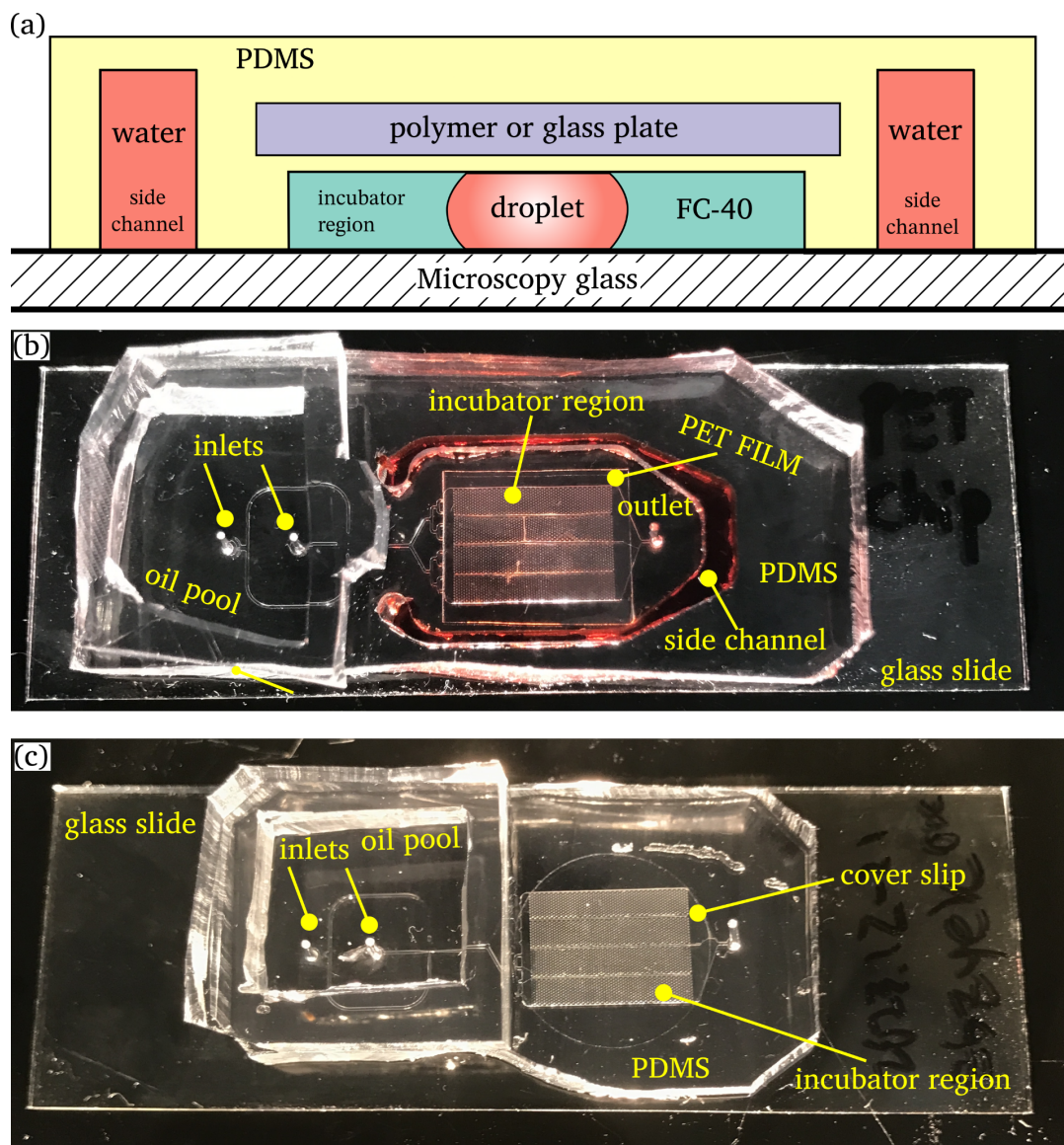


Fig. 3.10: (a) Schematic of the cross-section of the incubator region of the PDMS-glass chip with non-liquid permeable material insert and side channel. (b) The actual image of the chip with PET film and side channel filled with red-colored water. (c) The actual image of the chip with circular microscopy glass coverslip buried inside the PDMS.

(see Fig. 3.11c). Rubber plugs were added to the inlet and outlet ports to hold the tubing (see Fig. 3.11c). These plugs, made from PDMS, are slightly thicker than the depth of the inlet and outlet ports in the top plate (see Fig. 3.11d). When compressed, each plug forms a seal between the glass plate and the exterior, as well as between the tubing and the exterior. Finally, all components were assembled using nine M2 machine screws, tightened with a torque screwdriver set to approximately 0.2 Nm (Proxxon Microclick). The assembled constructible chip is shown in Fig. 3.11e. After testing, the chip was confirmed to be leak-free. Unlike other chips used in this research, this chip has the advantage of allowing complete disassembly, enabling each component to be washed properly.

Note that the glass-glass chip includes only the incubator region and does not contain the droplet generator. A separate PDMS-glass chip with a microfluidic cross-junction was prepared, with its outlet connected to the inlet of the glass-glass chip, as shown in Figs. 3.11b and e. Despite the use of two chips in the system, all experimental procedures, such as medium loading and the

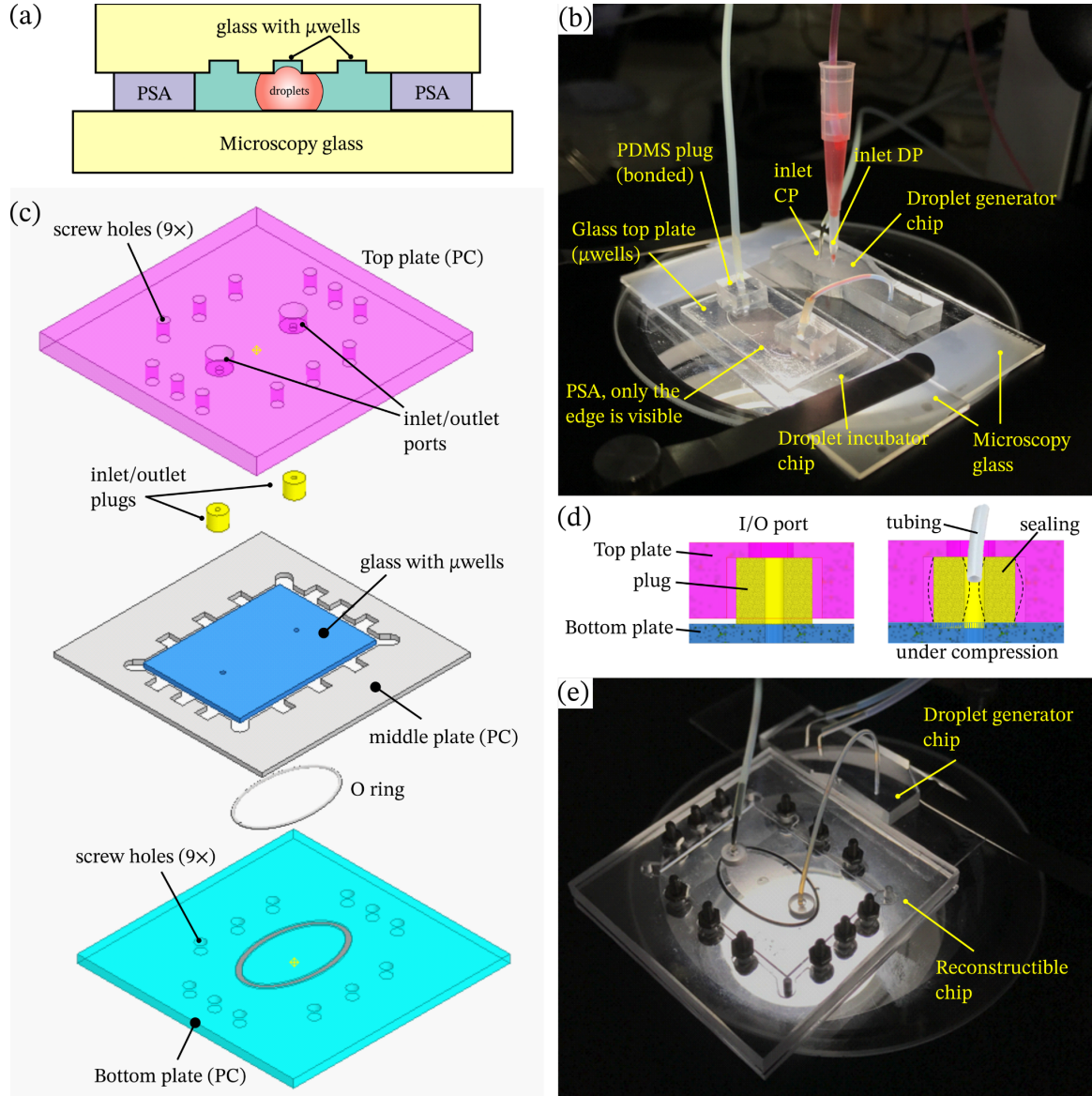


Fig. 3.11: The glass-glass chip. (a) Schematic and (b) the actual image of the glass-glass chip assembled using PSA tapes. (c) The components, and (d) the actual image of the constructible glass-glass chip.

negative pressure driving system, remain the same as in the previous PDMS-glass chip setup.

3.4.4 The biological experiment

The experiment was performed at a controlled temperature and CO₂ level of 37°C and 5%, respectively. The imaging was performed using the Leica TIRF microscope. The typical time from cell loading until the droplet fully occupies the incubator is typically less than 10 minutes. The recording of cell interaction was performed for typically 24 hours.

3.5 Measurement of various parameters through image analysis

3.5.1 Image acquisition

A stereo microscope (Zeiss Stemi 508) equipped with a CCD camera (IDS UI-3274LE-C-HQ) was used to observe droplet formation. In the high temporal and spatial resolution experiment

that was intended to observe the formation process in the junction, the recording frame rate was set to 45 fps. For experiments to measure droplet length and observation of various flow patterns, a frame rate between 1–20 fps is enough, depending on the flow rate of DP. In the device with a high aspect ratio and very low flow rate (very low Ca values), the period between droplet pinch-off can be longer than 30 minutes, or in other words, around one hour per droplet. Thus, low frame rate recording reduces video file size and does not harm the measurement in the simple experiment.

3.5.2 General image processing procedure

Recorded images were analyzed by a script written in the Python 3 programming language. The script uses available image-processing packages such as Scikit-Image [98] and Scipy [99]. These packages were built on top of Numpy, the fundamental library of scientific computing in Python [100]. Numpy is a library that provides multidimensional array objects and the tools for manipulating such arrays. An image is an array object that contains a collection of numbers constructed in a grid. Each number represents the intensity of an image in a particular position. There are various number types representing the intensity; the most common types are the integer number between 0 (black) – 255 (maximum), the floating number between 0 and 1, and the binary number either 0 (False) or 1 (True). For example, an RGB image is an array of numbers in three-dimensional “construction” with a size that can be written using a tuple (M, N, 3). M and N represent what we understand as the size of a photo, height (number of rows, M) $\times$ width (number of columns, N), in pixels. The last entry of the tuple represents the total number of layers in the image. (M, N, 1) image is the layer green of an RGB image with the size M $\times$ N pixel. Python was chosen because it is a high-level and open-source general programming language, such that the packages are available for free. The typical droplet image analysis procedure (algorithms) is as follows:

1. If the background image is available, the interface of a forming droplet (or a droplet) can be extracted from an image by subtracting the intensity of the droplet-containing image from the background image. Then take the absolute value of intensity in every pixel. The resulting image is a grayscale image consisting of the interface of the droplet only.
2. If the background image is not available, a background image can be produced by another image processing. In the case of a bright field image with a uniform light coming from the back of the chip, creating a background image (i.e., the image consisting of empty microchannels) can be done by taking the maximum intensity from many images with different positions of droplets. Since the interface of a droplet is a shadow (low-intensity value), the interface will be replaced with higher-intensity pixels from other images.
3. The grayscale droplet image is then converted into a binary image by setting the threshold value.
4. Reducing the noise by executing one or combinations of morphological operations such as erosion, dilation, opening, closing, and removal of small objects.
5. At this stage, the resulting image contains a droplet outline, and measurement of various parameters can be performed. If required, the area enclosed by the outline can be filled, that is, by replacing the zero values within the outline with the binary value of 1, as shown in Fig. 3.12c.

The exact implementation of the algorithms above depends on the quality of recorded images. Various methods, such as the Hough transform for automatic detection of the circular edge or Canny filtering for automatic edge detection, can be applied. An example of basic measurement will be shown next.

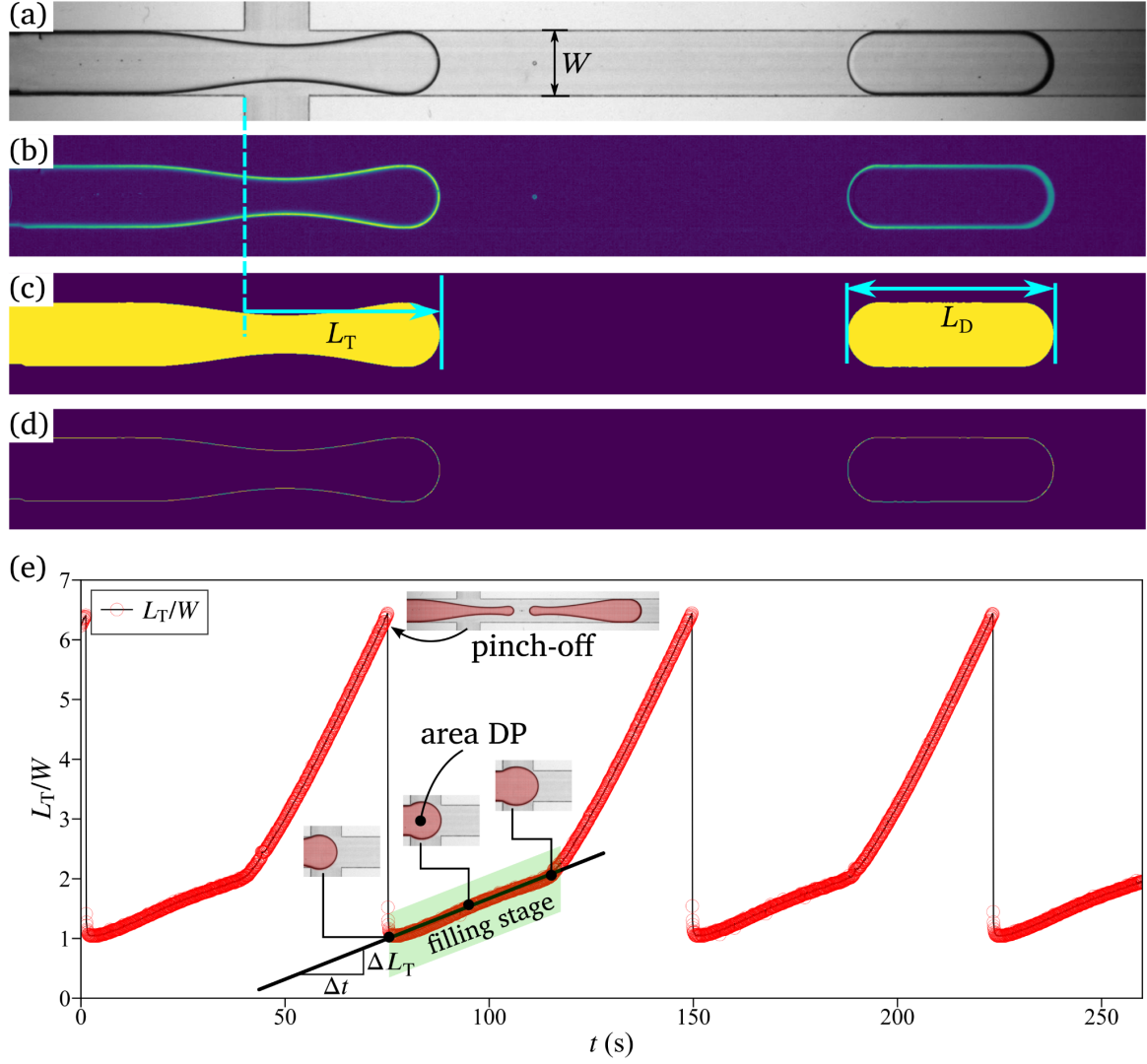


Fig. 3.12: An example of image processing result: (a) Original grayscale image (b) image containing the droplet shadow only as a result of subtraction with the background image. (c) An image containing a filled droplet. This kind of image is useful for measuring the area of droplets. (d) An image containing an outline of droplets is useful for measuring parameters obtained from the interface. (e) Measurement result of L_T versus time. The three images with shaded droplet area in the filling stage show the rate of growth of a droplet which is equal to the DP input flow rate, Q_D .

3.5.3 Measurement of the droplet length and the evolution of the forming-droplet

An illustration of the image analysis for the measurement of droplet length L_D and the tip position L_T relative to the cross-junction is shown in Fig. 3.12. The top image (Fig. 3.12a) shows the original image in grayscale. The next image shows a grayscale image as the result of subtracting the original image from the background image, i.e., the image only contains the droplets. Then, this image is converted to a binary image either with the filled holes (Fig. 3.12c) or a single-pixel outline (Fig. 3.12d), depending on the requirement of the measurement. Noise reduction procedures can be performed at any stage of the image processing; however, it is mostly after the subtraction of the image (Fig. 3.12b) or after the conversion to a binary image (Fig. 3.12c).

An example of the resulting measurement of L_T is shown as the red circles in Figure 8e. The

jump of L_T values marks the pinch-off. Following the pinch-off, the DP recedes into the cross junction and starts to grow with the rate proportional to the filling rate of DP, i.e., $\frac{dL_T}{dt} \propto \frac{Q_D}{HW}$. More accurately, the filling rate of the DP can be approximated by measuring the area within the growing droplet over time (see images with overlay of red color). The rate of change of the droplet area is approximately equal to Q_D/H . This method can be useful for the examination of the correctness of the DP pump flow rate, Q_D .

3.5.4 Measurement of the necking time and tip position at the start of the necking stage.

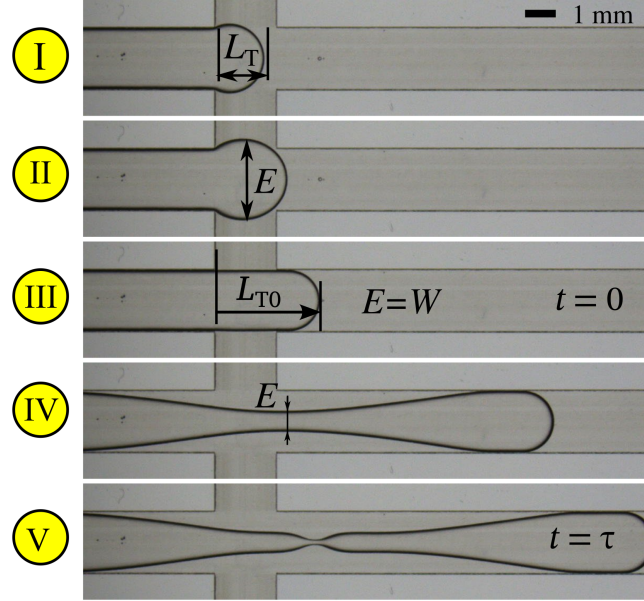


Fig. 3.13: Image sequence showing droplet formation and some quantities measured from the images.

Some parameters were obtained from a specific moment during droplet formation. Fig. 3.13 shows an image sequence of a single cycle of droplet formation with some measured quantities. L_T , as mentioned before, is the position of the droplet tip relative to the junction. E is the local maxima or minima of the forming droplet. The filling stage of droplet formation is the stage where the droplet grows (images I–III) before it occupies the whole junction and obstructs the outlet channel. The condition in image III, where $E = W$, is the moment where the beginning of the necking stage and the droplet-tip position at this time is denoted as L_{T0} . The necking stage ends with the pinch-off (image V), the time between image III and image V is the necking time, τ , which is one of the main quantities discussed in this research.

3.5.5 Measurement of the forming-droplet interface

The objective of the forming-droplets experiment was to observe the necking process in detail and extract some quantities from the images. Therefore, the recordings were made with a high frame rate (45 fps) and with the field of view focused only on the area of necking. An example of the recorded experiment image is shown in Fig. 3.14a. The outline of the droplets was obtained with the method described previously, and the example of a half-top droplet outline is plotted in Figure 10b as the black staircase-like line. For clarity, Fig. 3.14b is not plotted with an equal aspect ratio between the two axes, even though both axes have the same dimension, pixel (px). The resulting staircase outline was obtained because the image's spatial resolution is not high enough. The staircase signal creates additional noise for the measurement of some quantities.

The simplest measurement was the measurement of the maxima of the neck shape, denoted as A . A is a time-dependent factor that later will be used as the scaling factor in the direction perpendicular to the axis of the main channel. The initial measurement A resulting from maxima detection also shows a staircase-like curve (see squares in Fig. 3.14c). To overcome this problem, a quadratic fitting was used on the “top” of the neck. At first, obtain A and its coordinate on the image (see $\times$ in Fig. 3.14b). Second, take some data on the vicinity of the peak. This is done by cropping the data (x, y) near the peak based on the location of maxima to its left and right (in x -direction only) with a constant distance, typically 80 to 100 px. Alternatively, it is possible to crop the data based on the y value, e.g. using a threshold value $y \geq 0.95A$. However, the latter method does not always give better results compared to the former method because A changes significantly from near zero in the beginning and a much higher value near the pinch-off. The truncated data (using x -distance cropping) is shown as the red thick lines on the peak of the neck in Fig. 3.14b. The resulting measurement of A over time is shown as the black curve in Fig. 3.14c.

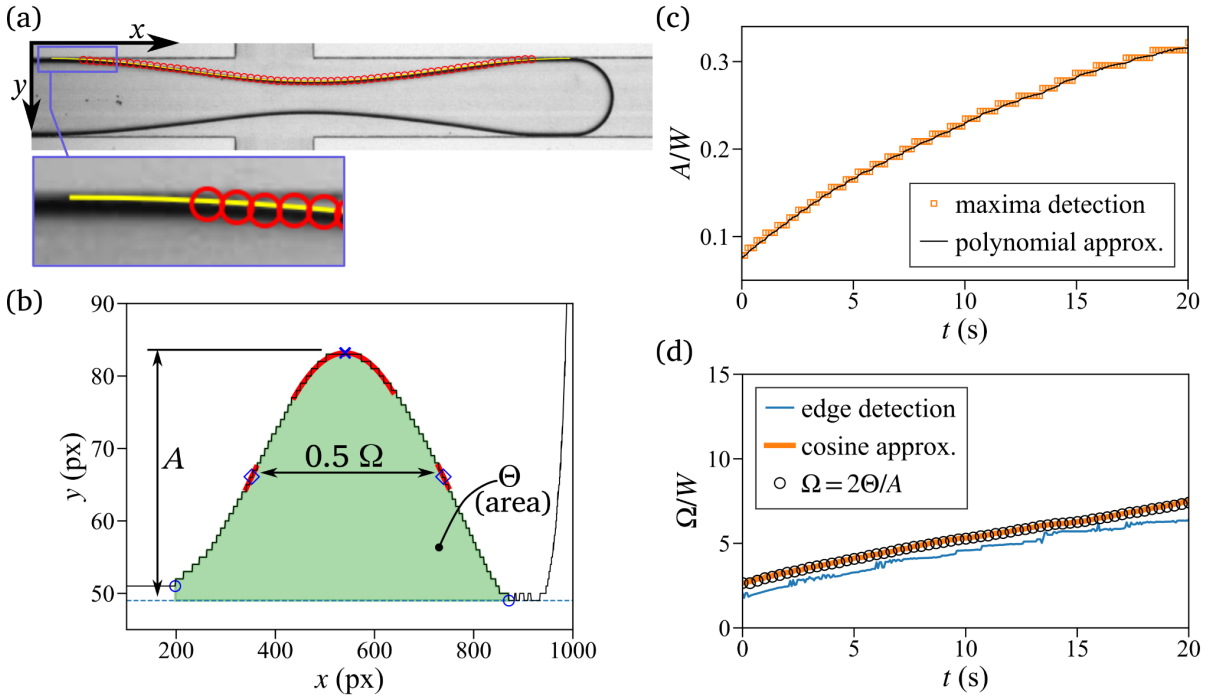


Fig. 3.14: Forming-droplet profile measurement (a) an example of neck profile during droplet formation. The red circles show the profile based on edge detection. The yellow line shows the approximation of the neck profile with a cosine wave shape. Inset: A dark area near the edge causes a too “early” edge detection, resulting in an overall shorter Ω (b) plot of the neck profile that shows the staircase-like curve. The thick-red lines show the local smoothing of the staircase data. The shaded green area is the area under the curve. Measurement result of (c) A and (d) D normalized with the width of the channel W

As a note, this method seems trivial since there are various mathematical methods for smoothing directly the curve of A vs t , such as convolution operation (for example, the standard “moving average” curve-fitting in MS Excel or LO Calc), polynomial regression, and many other methods. However, these curve-smoothing methods are best for finding the best fit/trend for noisy data or finding the data outliers, which means the resulting (smoothed) data will be “shaped” according to the function used for smoothing. Thus, it may alter the real evolution of A vs. t , which must be avoided at this stage since the function $A(t)$ is unknown. Even with the simple convolution operation, the window size (i.e., the span of data taken as the average for each point) will determine the shape of the curve A vs. t . This is due to the non-linearity of A vs. t . The quadratic

fitting at the top of the neck-curved used in this research is only to approximate the real value of A for each image, due to limited image spatial resolution. It does not change the evolutionary trend of A vs. t . Measurement of the neck width is more challenging. Not only it inherits the same staircase problem as A , but it has difficulty determining the correct end point of the neck shape. It is common to assume the end of the neck is a contact point between the solid wall, CP, and DP, as shown in the planar image (Fig. 3.14a top). However, there is no contact point between the DP and the wall because the DP does not wet the wall. The interface of the neck is smoothly approaching the wall until some part of it becomes parallel with the wall. The small gap between the smooth-approaching interface and the wall usually appears dark. This dark gap creates measurement uncertainty in the area near both ends of the neck, for example, the rugged outline near the RHS edge of the curve in Fig. 3.14b. The resulting measurement of the neck width Ω is rugged and fluctuates as shown as the blue line in Fig. 3.14d. As a remedy to the measurement problem of Ω , the shape of the neck is approximated as a part of a cosine wave as follows

$$h(x) = 0.5A \left[1 + \cos \left(2\pi \frac{x}{\Omega} \right) \right]. \quad (3.1)$$

$h(x)$ represents $y(x) - y_{\text{wall}}$, where y_{wall} is the y-coordinate of the channel wall as shown as the blue dashed line in Figure 11b. x in Eq. 3.1 is relative to the x-coordinate of the maxima of the curve A . The curve-fitting of Eq. 3.1 to the data shows a good fit, for example, as shown as the yellow line in Fig. 3.14a. Since the cosine wave has a symmetrical shape, measurement of the wavelength can be done by measuring the maximum -distance of the top-half of the neck profile. This is equal to the half of the wavelength, 0.5Ω , as shown in Fig. 3.14b. To obtain a more accurate middle points ($\diamond$ in Fig. 3.14b), linear regression is needed to flatten staircase data, as shown as two short red lines in Fig. 3.14b. Thus, there is no need to measure the edge of the neck profile, which is prone to error. The resulting Ω by cosine wave approximation is almost free of noise, as shown by the orange-thick line in Fig. 3.14b. Compared to Ω by edge detection (blue line), Ω by cosine approximation is slightly higher. This is because the dark area near the edge of the neck profile deems an “early” detection of the edge (see red $\circ$ in the inset in Fig. 3.14a), causing an overall shorter Ω as shown in Fig. 3.14a.

Another way to verify the correctness of the measurement Ω is by using the area under the curve, Θ , as shown by the shaded (green) area in Fig. 3.14b. Θ was measured by the trapezoidal rule (numerical integration) from the image of the neck profile. The detected edges were used as the left and right boundary of the area integration. Ω is then approximated by $\Omega = 2\Theta/A$ that is based on the integration of Eq. 3.1, $\int_{-0.5\Omega}^{0.5\Omega} h(x)dx = 0.5A\Omega$. The result is shown by the black $\circ$ symbols in Fig. 3.14d. The two measurements agree very well and the method by cosine approximation is used as the standard measurement method in this research.

3.5.6 The measurement for the relaxation of neck-shape after stopping the flow

The study of droplet formation also includes a special experiment in which the neck shape evolution after pinch-off is observed. The experiments were carried out as follows. First, the entire outlet channel was filled with DP, and then the DP flow was stopped. Second, the CP was supplied at a fixed flow rate to squeeze the DP in the junction. This started the necking process, which was similar to the droplet-formation process. Third, the CP flow was stopped before the pinch-off, as shown in the top image in Fig. 3.15. Fourth, the relaxation of the interface profile over time observed, as illustrated by the bottom image in Fig. 3.15.

3.5.7 Droplets size measurement and cell detection

Droplets were detected in bright-field images with the use of the circular Hough transform. Detected droplets were tracked in between neighboring time points using a linear sum assignment

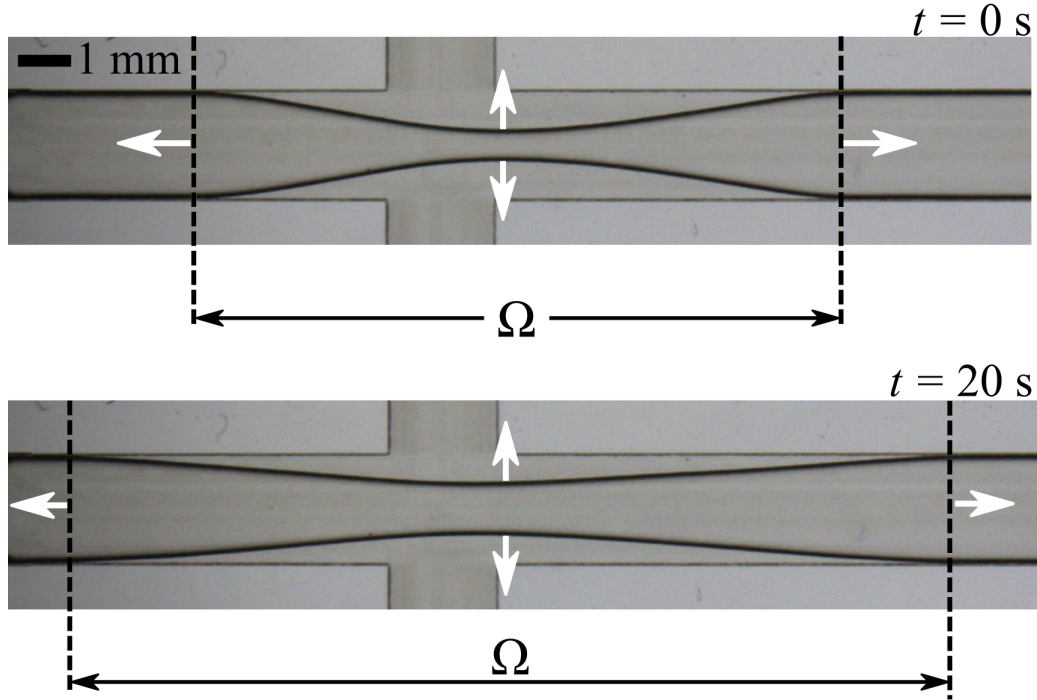


Fig. 3.15: The stop-flow experiments. Initially, the outlet channel is filled with DP, and then the DP flow is ceased. Then, the CP is fed with a constant flow rate Q_C . Lastly, the CP flow is stopped approximately before pinch-off, and the relaxation process is recorded and analyzed. Images of the neck shape immediately after stopping the flow (top) and 20 s later (bottom). Experiments were conducted in the cross-junction device with $W/H = 4.07$.

method utilizing both geometric and photometric features to assess droplet similarity. Droplet contents were determined by bright-field droplet image segmentation to find cell outlines and then the quantification of fluorescence intensities of cell dyes in these outlines. Segmentation has been performed by a U-Net-type deep convolutional neural network trained on manually annotated images of ~ 500 droplets containing ~ 1500 cells in total, with heavy augmentation. As a part of collaboration work, the image processing script was written using Python programming language by Dr. Marek Kohanczyk of the Institute of Fundamental Technological Research, Polish Academy of Sciences (IPPT-PAN).

3.5.8 Post-image processing data analysis

Data analysis and curve fitting were performed using the Scipy [99] and Lmfit [101] libraries. The resulting data were presented in color-deficiency-friendly plots using Matplotlib [102] and Cmasher [103] libraries.

Chapter 4

Results and discussion

4.1 Experiment results

4.1.1 Two-phase flow patterns at low Ca in microfluidic cross-junction device

Understanding the context of fluid flow in a microfluidic cross-junction is essential before jumping into the details of droplet formation. Therefore, this section presents general experimental results regarding various flow patterns emerging in the low-Ca range. As mentioned in the previous section, it is understood that multiple flow patterns emerge from two-phase flows through a microfluidic junction. The emergence of those flow patterns essentially depends on the geometry of the junction, the magnitudes of both liquid phase flows, the physical characteristics of liquids – viscosities, and interfacial tension.

Recall the two-phase flow through a cross-junction device, as shown in Fig. 2.8, the droplet phase (DP) flowing through the cross-junction with the rate Q_D and meets another flow, the continuous phase (CP), with the rate of Q_C . As discussed in the previous section, two-phase flow through microchannel is dominated by the viscous and surface tension forces, characterized by the relative magnitude between the two forces, i.e., the capillary number. The capillary number used in this study is the capillary number associated with the continuous phase flow, defined as:

$$Ca = \frac{\mu_C Q_C}{\gamma W H}, \quad (4.1)$$

where μ_C is the viscosity of the CP, γ is the interfacial tension, W , and H is the width and height of the rectangular cross-section channel.

In the low Ca range, droplet formation only occurs when the DP is large enough to occupy the junction, obstructing the way to the outlet channel. When the path to the outlet channel is obstructed, the CP is accumulated, allowing deformation (i.e., necking) of the DP. The necking process may result in the breakup of DP into droplets. The droplets produced in this low Ca range are larger than the width of the channel. In the higher Ca range, the CP is able to deform the DP without the DP occupying the outlet channel. Deformation predominantly occurs because of the high viscous drag of the CP. Droplets formed in this mechanism are smaller than the width of the channel, which is known as dripping or jetting flow (see Fig. 2.6), and the droplet formation is inherently sensitive to Ca and the properties of the liquid (viscosity of each liquid). This study focuses on the low-Ca regime, commonly known as the squeezing regime. The range of Ca included in this study is less than a few 10^{-3} .

Within the low Ca range mentioned above, various flow patterns over a wide range of Q_D and Q_C were recorded, and the result is presented as a flow pattern map for each device, as shown in Fig. 4.1. There were four different flow patterns observed, which the occurrence depends on the flow rate ratio, $q = Q_D/Q_C$ and the geometry of the channel, i.e., the channel aspect ratio W/H . The four flow patterns are:

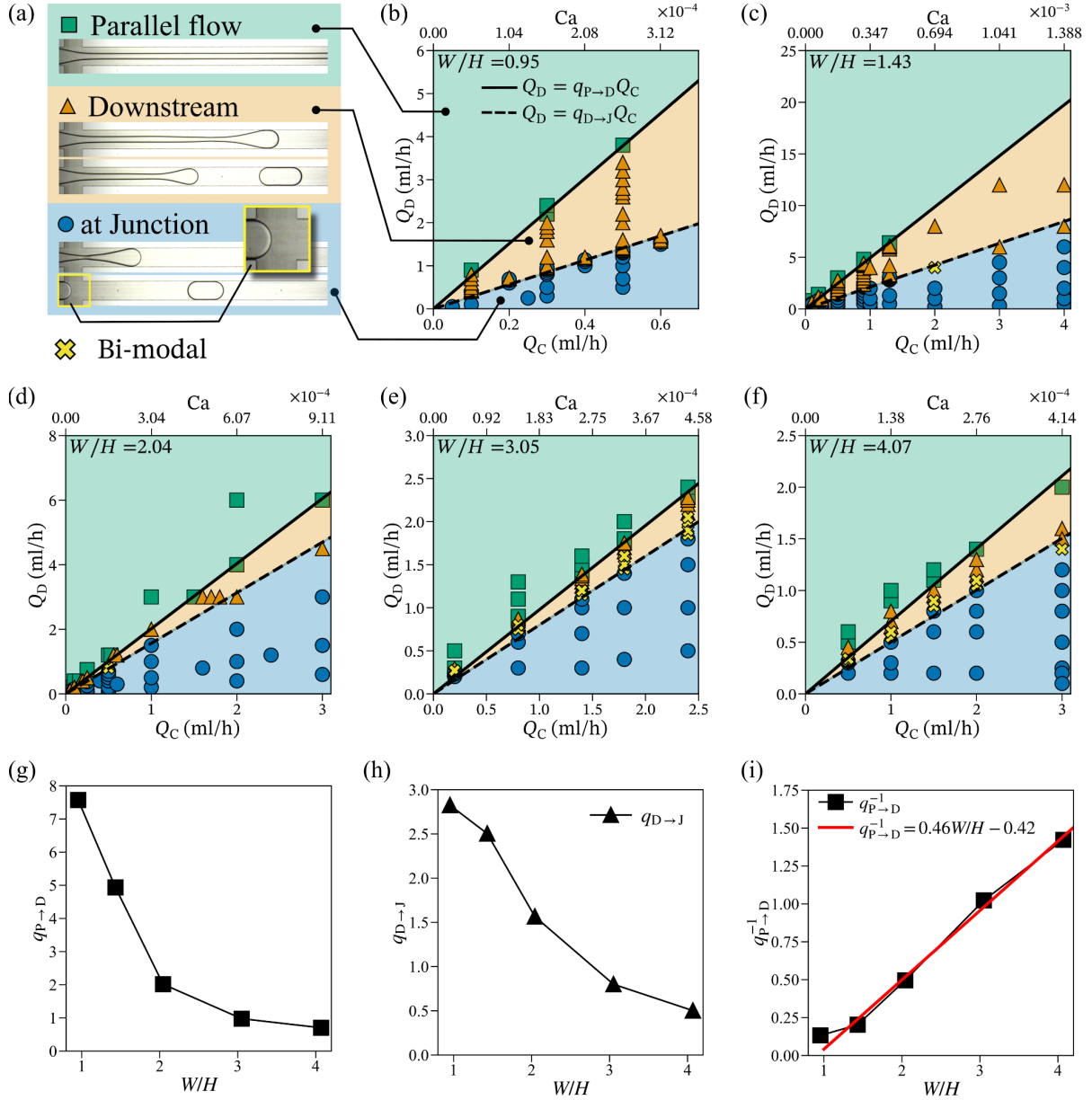


Fig. 4.1: Observation of flow patterns at low Ca flow regime (a) The flow pattern emerged due to different flow rate ratios. (b)-(f) flow patterns map for different device aspect ratios. (g) $q_{P→D}$ and (h) $q_{D→J}$ decrease against W/H (i) the linear proportionality of the inverse of $q_{P→D}$ against W/H .

1. **at Junction.** When q is quite low, the CP deforms the DP pretty quickly, resulting in the location of the pinch-off (i.e., the breakup of the DP) occurring in the vicinity of the junction. The DP thread, i.e., the DP still connected to the supply line, recedes back to the junction or inlet channel (see blue highlighted in Fig. 4.1a). Hence, the name “at Junction.” The at-Junction mode is shown as the blue circles and regions in Figs. 4.1a-f.
2. **Downstream.** Downstream mode, which occurs at intermediate q , is the droplet formation that its pinch-off location occurs quite far downstream of the outlet channel (see yellow highlighted in Fig. 4.1a). After the pinch-off, the receding DP thread stays inside the outlet channel and immediately blocks the channel. The location of the pinch-off and, consequently, the receding DP thread cause a fundamentally different droplet formation process compared to the at-Junction mode, which will be described in the next subsection.

The Downstream mode is shown as the orange triangles and regions in Figs. 4.1a–f.

3. **Parallel flow.** At higher q , the rate of CP flow is not high enough to allow deformation of DP that may trigger the pinch-off. Instead, the CP is flowing side by side with the DP, creating a stable filament, hence the name “Parallel Flow.” The parallel flow mode is shown in the green squares and regions in Figs. 4.1a–f.
4. **Bi-modal.** The Bi-modal flow pattern is a special pattern where both at-Junction and Downstream modes appear. The cycle between two (or more) modes creates droplets with two (or more) distinct sizes. This pattern appears between the at-junction and downstream regions and is mostly observed only in cross-junctions with W/H larger than two. The Bi-modal mode is shown as the yellow X points in Figs. 4.1c–f.

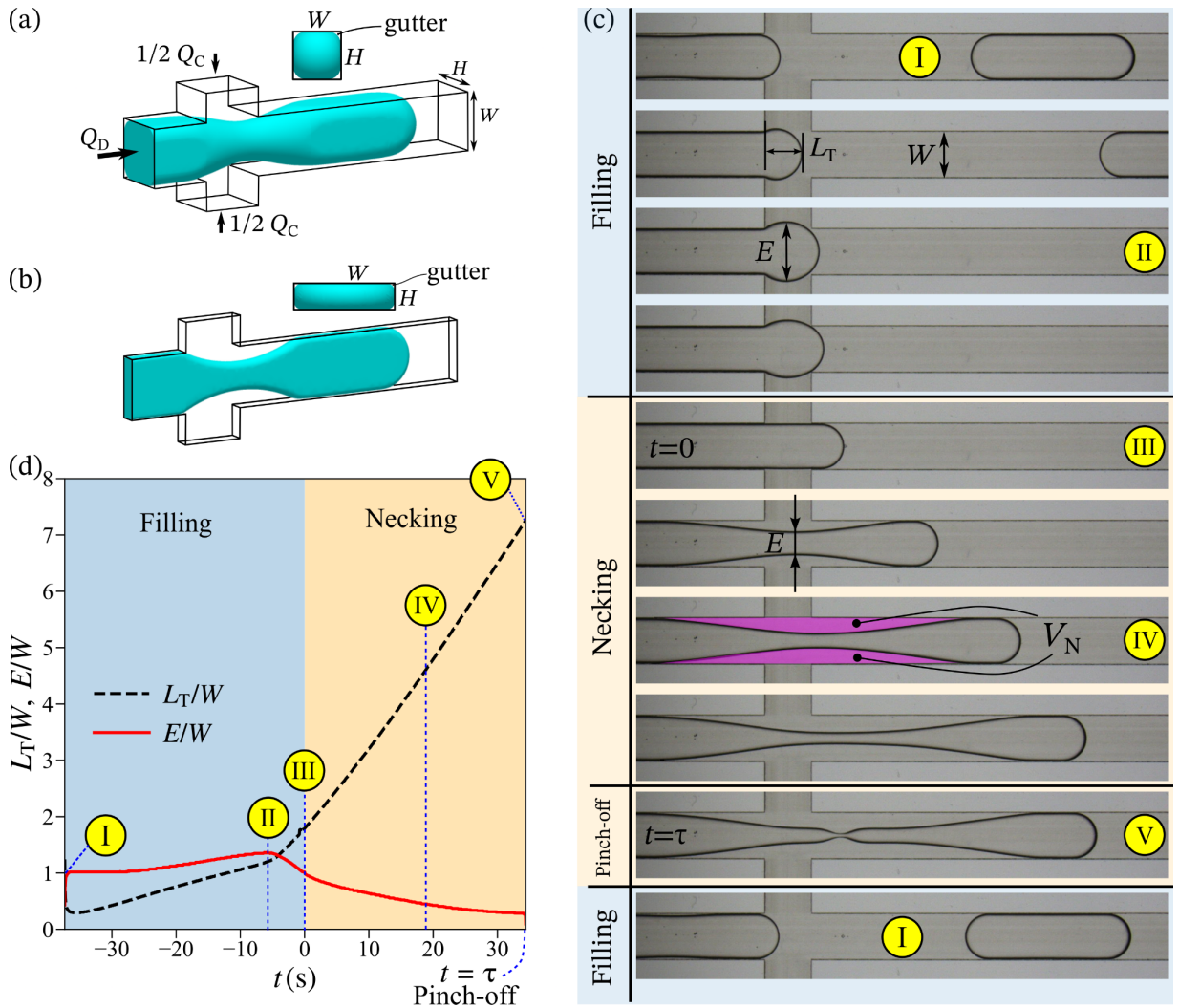


Fig. 4.2: 3D illustration of droplet formation in a cross-junction with W/H (a) 1 and (b) 4. (c) at-Junction droplet formation stages: filling, necking, and pinch-off. (d) the droplet tip L_T and local maxima/minima droplet with E evolutions of a single droplet formation cycle. The filling stage ends when the DP blocks the outlet channel and attains a straight droplet shape, $E = W$. The necking time is taken from the end of the filling stage ($t = 0$ s) up to pinch-off ($t = \tau$). The necking volume, V_N , is the growing volume surrounding the forming droplet, shown as the area with pink shade. Experimental images and measurements are taken from the device with $W/H = 4.07$. $Q_C = 1.00$ ml/h, $Q_D = 0.30$ ml/h.

Flow pattern maps in terms of Q_C and Q_D are shown for channels with different aspect ratios in Figs. 4.1b–f. While these maps show the emergence of each flow pattern that depends on q , the limit value of q of which a single pattern emerges depends on the device aspect ratio, W/H . These maps also show that the two transitions between the three flow patterns can be approximated by two straight lines, i.e., for constant q . The value of q that corresponds to the transition between "Parallel Flow" and "Downstream" droplet formation is defined as $q_{P \rightarrow D}$. Similarly, the value of q that corresponds to the transition between droplet formation "Downstream" (including the Bi-modal) and "At junction" is defined as $q_{D \rightarrow J}$. As shown in Figs. 4.1g and h, $q_{P \rightarrow D}$ and $q_{D \rightarrow J}$ are decreasing with the increasing W/H . The current result is in accordance with previous work in [104], where Parallel Flow is observed for a broader range of operating conditions in shallower channels. Following the arguments of Humphry et al.[105], the inverse of $q_{P \rightarrow D}$ can be approximated by a linear function of the aspect ratio for $W/H \gg 1$, i.e., $q_{P \rightarrow D}^{-1} = 0.46 \frac{W}{H} - 0.45$, with the coefficients obtained from a fit to the experimental data (see Fig. 4.1i).

4.1.1.1 Droplet formation — at-Junction

A three-dimensional image illustrating the droplet formation process in cross-junction with aspect ratio (W/H) equal to one and four are shown in Figs. 4.2a and b, respectively. The actual experimental images of at-Junction droplet formation are shown in Fig. 4.2c. From the image sequence, parameters such as the droplet tip length (L_T) and the local maxima and minima of the droplet (E) are measured and plotted over time (t), as shown in Fig. 4.2d. The At-junction droplet formation consists of three stages:

1. **Filling.** This is the stage when the DP is flowing through the junction and has not yet occupied the outlet channel. The CP flows through the junction and goes directly to the outlet channel with minimum interference from DP. Finally, the tip of DP is long enough to fill the outlet channel. The end of the filling stage is marked when the droplet becomes a straight plug, $E \approx W$, as shown by the marking II in Figs. 4.2c and d.
2. **Necking.** The time zero of the necking stage was taken when DP forms a straight plug, as shown in Fig. 4.2c. As the DP occupies largely the outlet channel and blocks the way for the CP, it increases the flow resistance for the CP flow passing through the droplet. Consequently, the CP accumulates upstream of the droplet tip, and it starts to squeeze the DP. This process is called necking (see IV in Fig. 4.2c). The necking process ends when the neck of the droplet is thin enough that it becomes unstable due to the so-called Plateau-Rayleigh instability.
3. **Pinch-off.** The instability drives the collapse of the thin neck, producing the droplet of DP (see V in Fig. 4.2c). The process occurs in a very short time and is called the pinch-off.

4.1.1.2 Droplet formation – Downstream

The Downstream droplet formation process is very similar to the at-Junction mode except that, because of the higher flow rate ratio, the necking occurs much further downstream of the junction. After the pinch-off, the droplet thread (i.e., the droplet phase that is part of the DP supply line) recedes and stops inside the outlet channel. Then the process is followed by the necking stage, skipping the filling stage. Thus, the Downstream droplet formation consists of only two stages: necking and pinch-off, as shown in Fig. 4.3.

4.1.1.3 Bi-modal droplet formation

The bi-modal droplet formation consists of two formation modes: at-Junction and Downstream. The two modes appear alternately, as shown in Fig. 4.4, producing two distinct droplet sizes. The

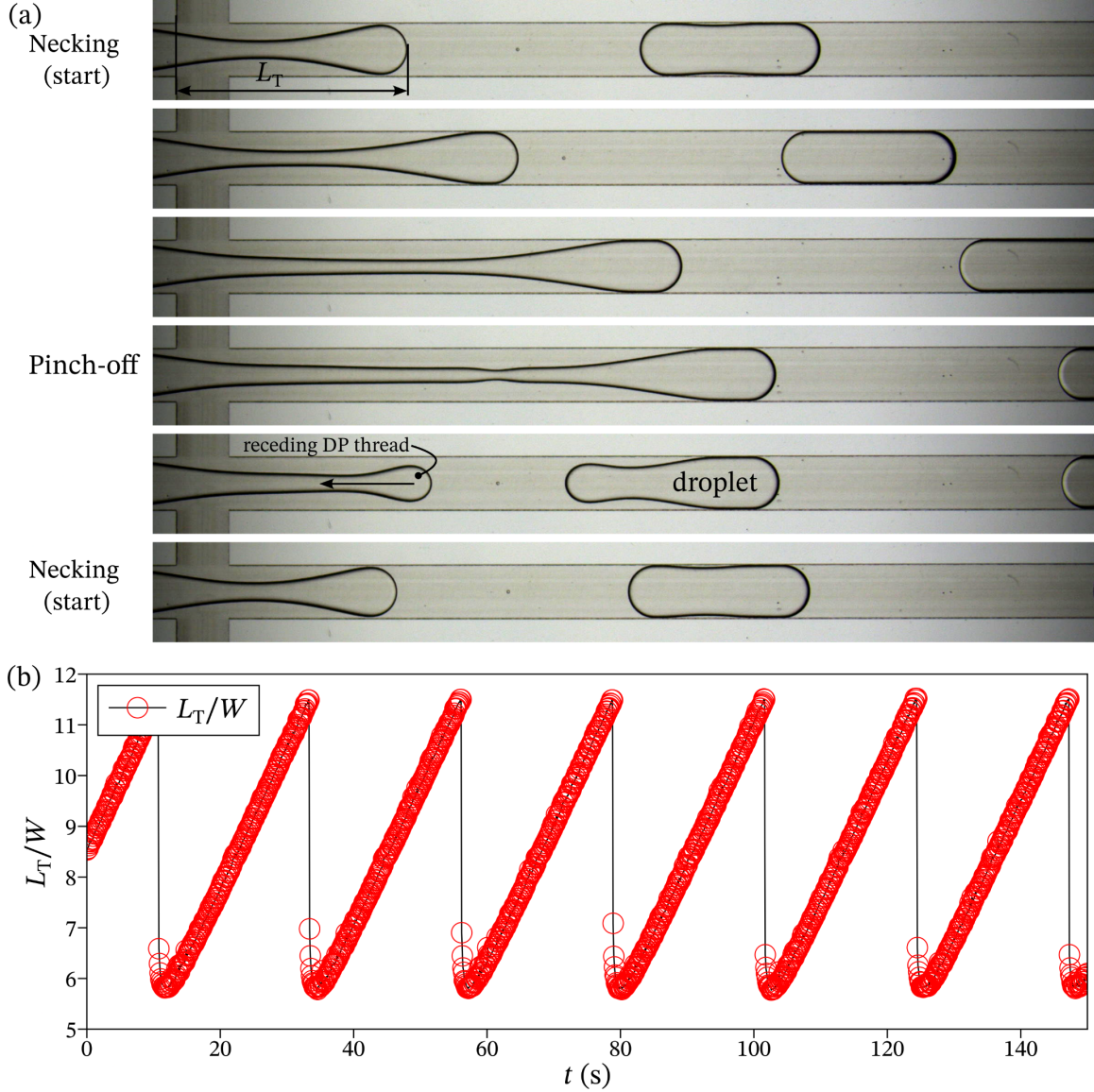


Fig. 4.3: (a) The downstream droplet formation occurs in just two stages: necking and pinch-off. (b) The droplet tip length cycle shows that the process begins with the relaxation of the DP thread, continues with the necking phase, and concludes with the pinch-off. Experimental images and measurements are taken from the device with $W/H = 4.07$. $Q_C = 1.50$ ml/h, $Q_D = 0.90$ ml/h.

droplet size made in the at-Junction mode is larger than the one produced in the Downstream mode. This argument is supported by the measurement of L_T as shown in Fig. 4.5a. The measurement indicates clearly that there are alternating modes in the process, with the green and brown regions showing the at-junction and downstream modes, respectively. The at-junction process takes a longer time—shown by the wider green area in Fig. 4.5a—because each cycle consists of filling and necking stages. Since the time for forming the droplet is longer, the size of the droplet produced in the at-Junction mode is larger. The downstream mode has only cycles of the necking stage. Thus, droplet size is smaller. The measurement shown in Fig. 4.5c shows the sequentially alternating of two droplet sizes. The key to the process that allows the alternating modes is there must be a sufficient length of droplet thread during the At junction droplet formation, such that the receding thread after the pinch-off stops inside the outlet channel, allowing the subsequent droplet formation in the downstream mode. However, the droplet thread (as a result of at-Junction mode) cannot be too long; otherwise, there can

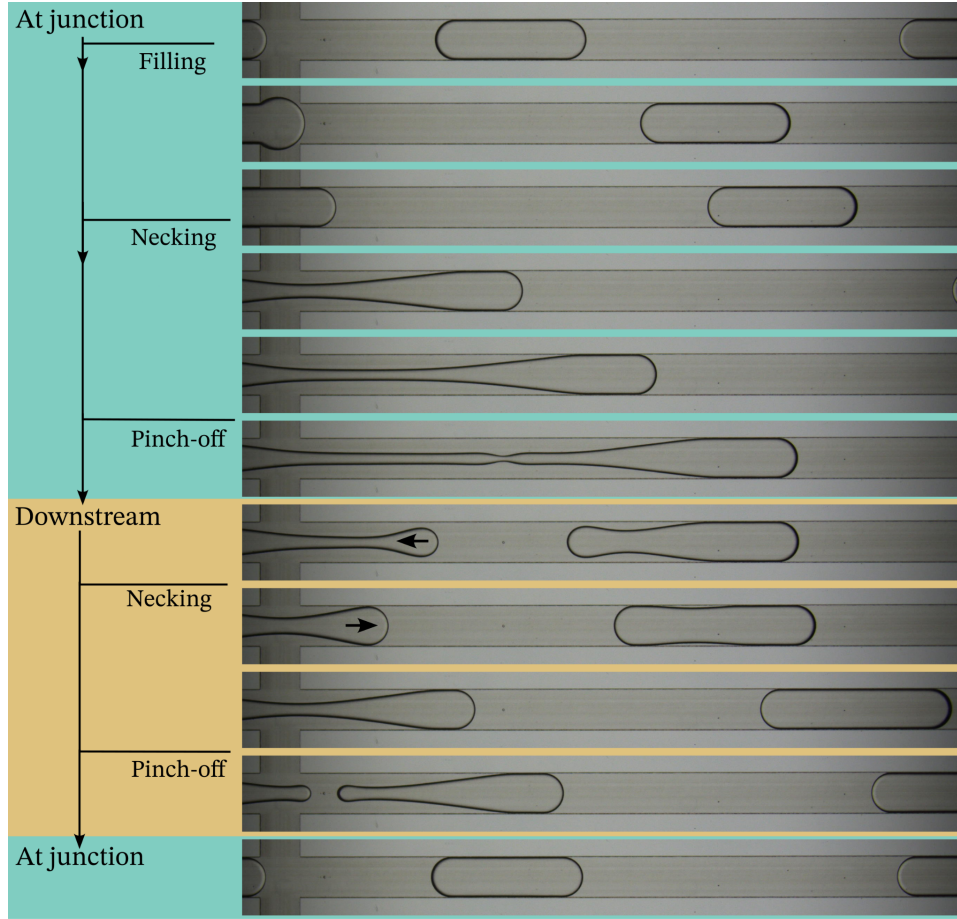


Fig. 4.4: The image sequence of one full cycle of bi-modal droplet formation. At junction (green) is followed by Downstream (brown), and then followed again by At junction (green). Images are taken from the device with $W/H = 4.07$ at $Q_C = 0.50$ ml/h and $Q_D = 0.20$ ml/h.

be only cycles of the Downstream droplet formation.

The focus of this work is on the droplet formation in low Ca . In the following sections, the analysis will be based on the at-Junction droplet formation.

4.1.2 The effect of capillary number and flow rate ratio on the droplet size

The fluid flow in a microfluidic junction is a complicated phenomenon which, as indicated above, depends on various factors such as flow conditions (q and Ca) and geometrical factors such as device aspect ratio (W/H). To investigate the effect of one flow parameter independently of the other, two different kinds of experiments were performed, each with one parameter kept as a constant while the other varied within the limit of droplet formation. Fig. 4.6a and Fig. 4.7a shows the experiment results with constant Ca and varied q for devices with W/H 1.43 and 4.07, respectively. The experimental images show that the dimensionless droplet volume, $l_D = L_D/W \approx V_D/HW^2$ increases linearly with the flow rate ratio, i.e., $l_D \propto q$. Indeed, a series of measurements from six different devices with various values of constant Ca shows the same linear proportionality between l_D and q , as shown in figs. 4.8(a)-(i). This linear proportionality has led to the classical squeezing scaling law, $l_D = c_1 + c_2q$, with both c_1 and c_2 as constants that depend on geometrical factors [30]. The classical squeezing law was a revelation by the time it was proposed, and it still holds true for the limited Ca regime. It states that the produced droplet length does not depend on the properties of the fluid, such as viscosities, nor does it depend on the magnitude of the viscous shearing forces and interfacial tension. It simply depends

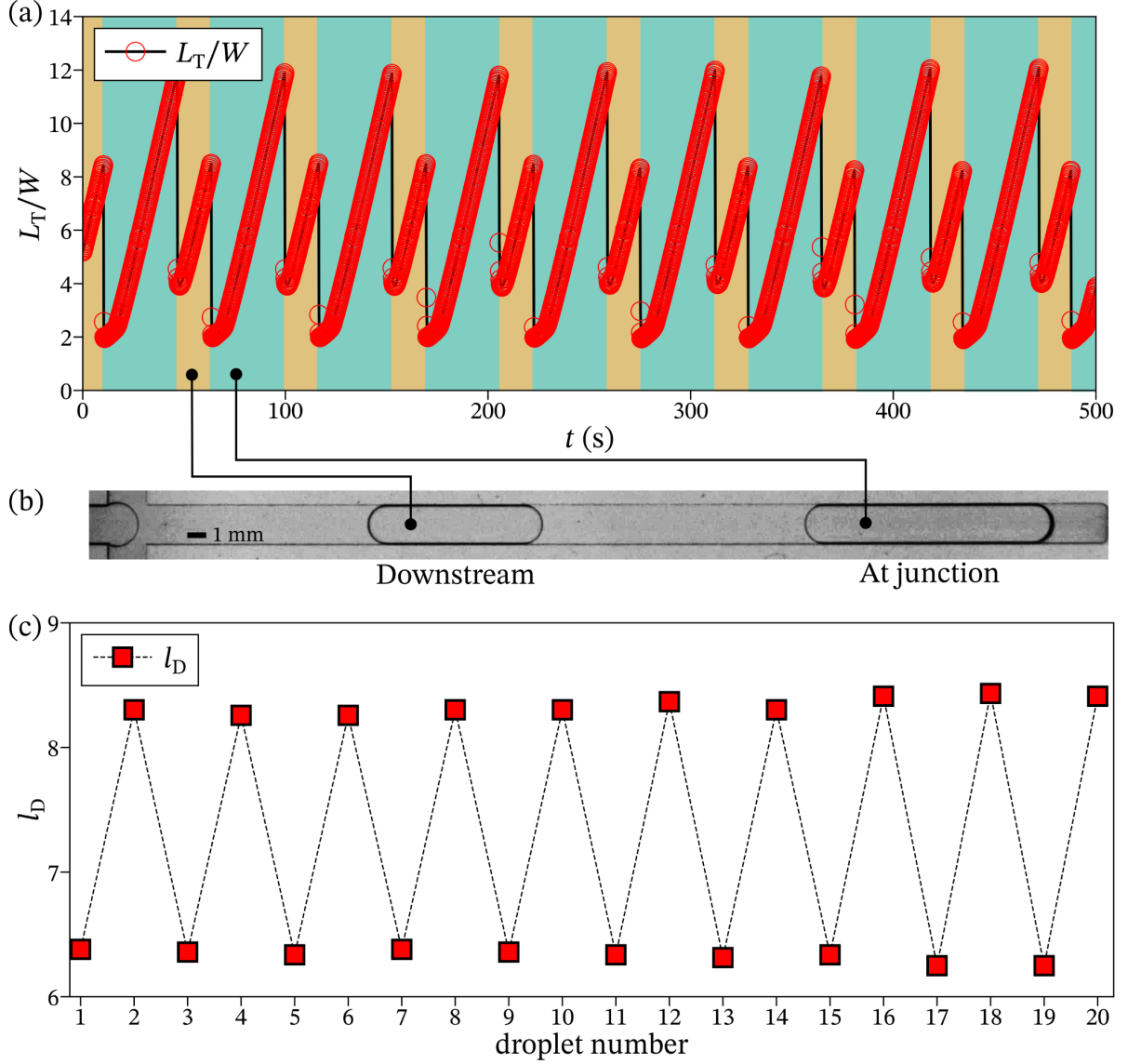


Fig. 4.5: a) The evolution of L_T shows two modes of droplet formation: at Junction (green) and Downstream (brown). (b) An experimental image shows the two distinct droplet sizes. (c) The sequential pattern of two different droplet sizes. Measurements are taken from the device with $W/H = 4.07$ at $Q_C = 1.50$ ml/h and $Q_D = 0.85$ ml/h.

on the geometry of the junction and the flow rate ratio. The explanation behind its simplicity relies on the filling and necking mechanisms, as described previously. It requires more or less the same volume of DP to obstruct the outlet channel, V_0 . Once the necking is started, the additional droplet volume depends on the speed of the necking process, that is $\propto q$. Accordingly, the classical squeezing law can be written as:

$$l_D = l_0 + v_{N0} q. \quad (4.2)$$

The classical scaling law (Eq. 4.2) was re-derived here based on the experimental evidence, and it requires further discussion. The first term, l_0 , is understood as the dimensionless DP volume that is required to block the outlet channel, which is assumed to be constant. The second term contains v_{N0} , i.e., the slope of the linear equation, which understood as the total volume of DP displaced by the volume under the neck (see pink shaded area, V_N , in Fig. 4.2c) [63, 54]. To see how the physical meaning of l_0 and v_{N0} come from, Eq. 2.15 is rewritten here as $V_D = V_0 + \tau Q_D$. The first terms of Eq. 4.2 and Eq. 2.15 are related by $l_0 = V_0/HW^2$,

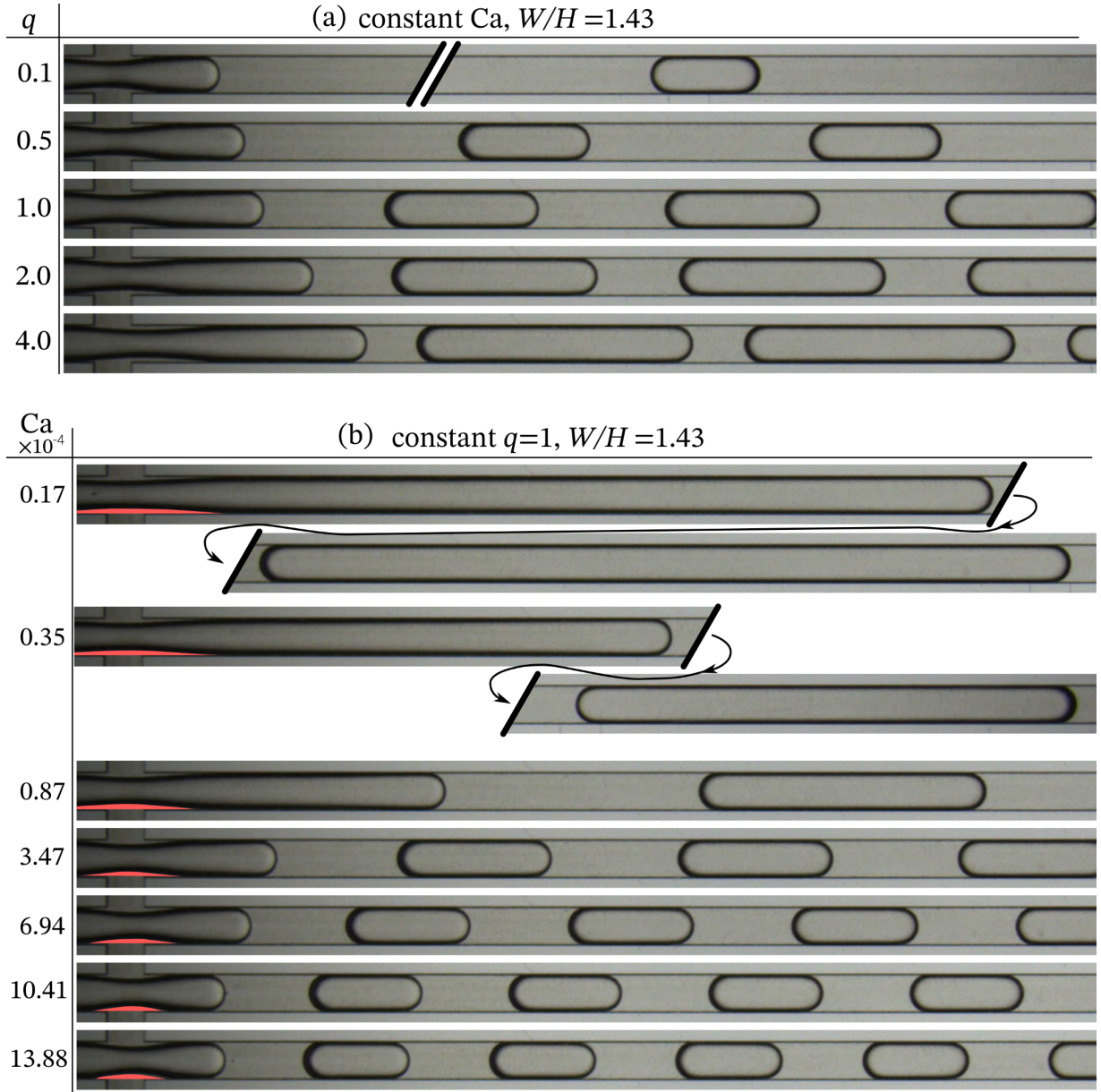


Fig. 4.6: Two series of experiments performed in different conditions: (a) constant Ca and varying q , (b) constant q and varying Ca . Experimental images are taken from the device with $W/H = 1.43$.

and it represents the volume of DP at the end of the filling stage (see Fig. 4.2c). During the necking stage, the droplet volume is increasing at the rate Q_D while being squeezed by the CP. If the rate of necking is assumed to be Q_C , then the total necking time, τ , can be estimated as $\tau = V_{N0}/Q_C$, where $V_{N0}(=V_N(\tau))$ is the volume under the neck shape at the end of the necking stage ($t = \tau$). Thus, with this assumption, the second term in Eq. 2.15 and Eq. 4.2 is related by $v_{N0} q = V_{N0} Q_D / (HW^2 Q_C)$. As the physical interpretation of the constant parameters in Eq. 4.2 has been clarified, the next step will be showing the fall of Eq. 4.2 against the experimental evidence.

Fitting Eq. 4.2 to each data set of constant Ca as shown in Fig. 4.8 shows that linearity of droplet size against the flow rate ratio stated by Eq. 4.2 is true. However, It is also noticeable there are some changes between the lines of different Ca . The intercepts and the slope of the lines increase with decreasing Ca value but in a non-linear manner. This non-linearity can be explained by observing the experimental results with a constant value of q and varying Ca , as

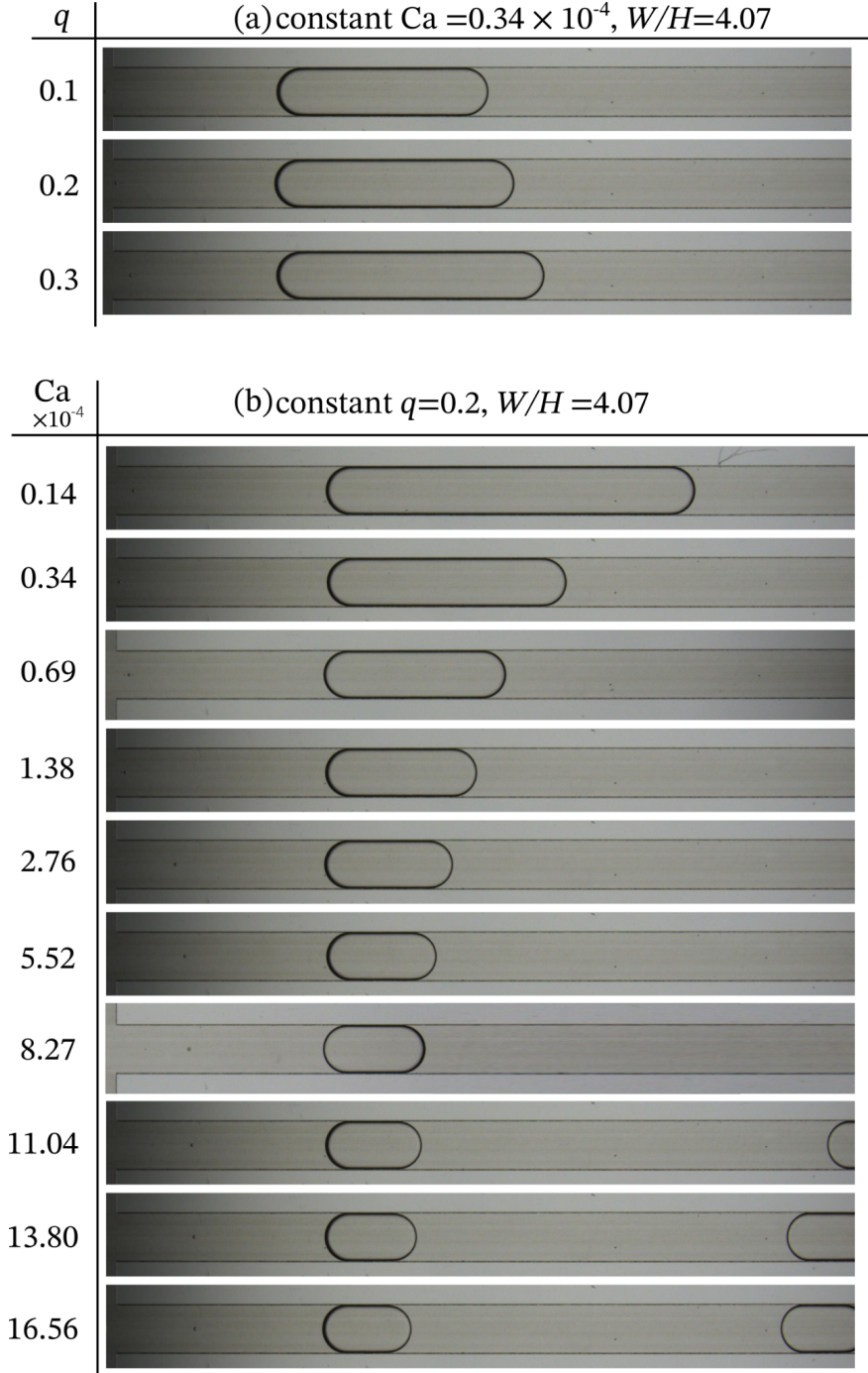


Fig. 4.7: Two series of experiments performed in different conditions: (a) constant Ca and varying q , (b) constant q and varying Ca . Experimental images are taken from the device with $W/H = 4.07$.

shown in Fig. 4.6b and Fig. 4.7b. At moderately high Ca , the droplet size is almost constant over some range of Ca , agreeing to the Eq. 4.2. However, for lower Ca values, there is an extreme increase of l_D over decreasing Ca . The measurement of l_D across five different rectangular cross-section devices also shows the same trend, as shown in Fig. 4.9. Thus, the current experimental results, as shown in Figs. 4.6 and 4.7 have indicated that either one or both coefficients in Eq. 4.2 has dependency to Ca . The sudden increase of l_D at very low Ca in the current experiment is similar to the previously described leaking regime at the T-junction [60] (see Section 2.4.2). The key point of leaking flow was that not all CP contributes to the necking process; instead,

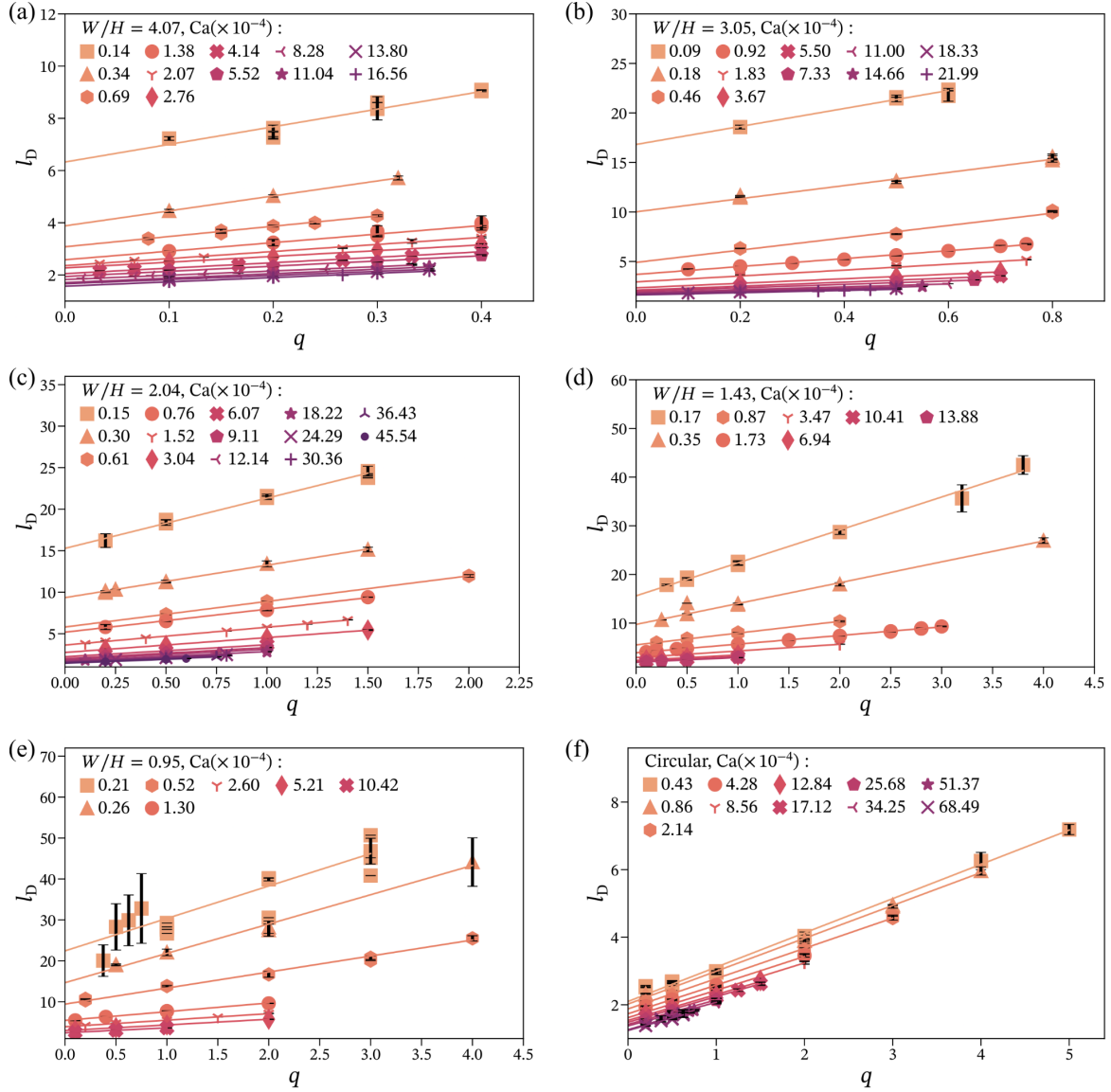


Fig. 4.8: Dimensionless droplet length versus flow rate ratio for (a)-(e) five rectangular cross-section devices and (f) one circular device. The points are the measurement result, whereas the solid lines are the linear lines based on the curve-fitting to each data set at constant Ca .

some of it flows through the gutter. Could the same leaking mechanism in the T-junction apply in the cross-junction device? The experimental results (Fig. 4.8 and Fig. 4.9) show that the increase of l_D at decreasing Ca only occurs in the cross-junctions with rectangular cross-section, suggesting that the leaking flow through the gutter causes the great increase of l_D at very low Ca .

To answer this question and to investigate the droplet formation at very low Ca , it is needed to discard the assumptions that lead to Eq. 4.2 and include the current experimental findings:

- The intercept and slope of the fitting lines, as shown in Fig. 4.8, increase against the decreasing Ca , except for the case of circular cross-section device (Fig. 4.8f).
- The increase in l_D at very low Ca could be due to the leaking flow. Thus, it can be assumed that only a fraction of the CP contributes to the necking, defined as $q_N = Q_N/Q_C$. Q_N is the flow rate that fills the volume under the neck, V_N .

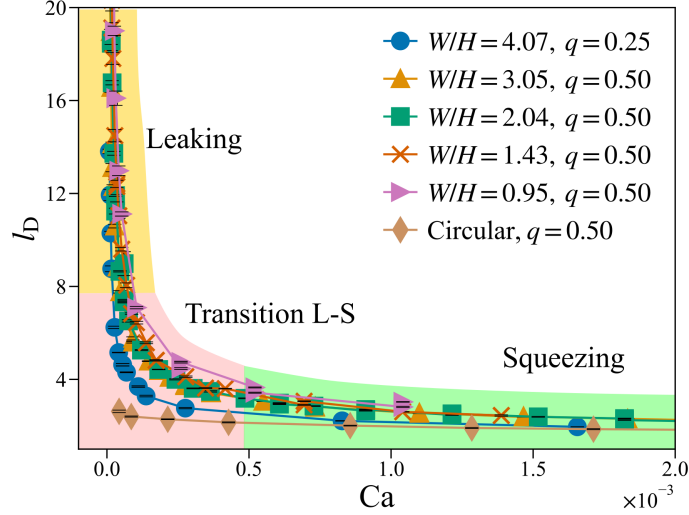


Fig. 4.9: Dimensionless droplet length versus Ca for selected values of q . Measurements were obtained from five rectangular cross-section devices and one circular cross-section device.

The linearity of droplet size to the flow rate ratio has been shown valid; thus, it can be used for derivation of the general scaling law. The derivation is started from the relation, $V_D = V_0 + Q_D \tau$. The intercept of the fitting lines in Fig. 4.8a–e is l_0 , which is the dimensionless form of V_0 . Due to the second argument above, τ in of the second term is redefined as $\tau = V_{N0} / \langle Q_N \rangle$, where $\langle Q_N \rangle$ is the time-averaged necking flow rate. Note that at this point, the term $\tau = V_{N0} / \langle Q_N \rangle$ is not equivalent to the second term in Eq. 4.2 because $\langle Q_N \rangle \neq Q_C$. Thus, the second term can be written as $\tau Q_D = \frac{V_{N0}}{\langle Q_N \rangle} Q_D$. Defining dimensionless necking time as $\tau^* = \tau Q_C / HW^2$ and the time-averaged fraction of CP that contributes to the necking as $\langle q_N \rangle = \langle Q_N \rangle / Q_C$, the second term can be written in dimensionless form as $\tau^* q = \left(\frac{V_{N0}}{HW^2} \right) \frac{q}{\langle q_N \rangle}$. Finally, the generalized form of the scaling law for dimensionless droplet volume can be written as:

$$l_D = l_0 + \tau^* q. \quad (4.3)$$

Note that $\tau^* = \tau \frac{Q_C}{HW^2} = \left(\frac{V_{N0}}{\langle Q_N \rangle} \right) \frac{Q_C}{HW^2} = \frac{v_{N0}}{\langle q_N \rangle}$. Although Eq. 4.3 still assumes the linear relationship between l_D to q , its parameters, l_0 and τ^* , are no longer assumed as constants. In the higher Ca range such as shown in Fig. 4.6b and Fig. 4.7b where the droplet size is independent to the Ca , l_0 can be assumed as constant and the leaking flow can be assumed as negligible, $\langle q_N \rangle = 1$. In this case, Eq. 4.3 returns to the classical scaling law, Eq. 4.2. In the following sections, mathematical analysis will be developed to determine the dependency of l_0 and τ^* to the capillary number.

4.1.3 The increase in necking time, initial volume, and necking volume at very low Capillary numbers.

The intercept and the slope of fitting lines in Figs. 4.8a–f can be interpreted as l_0 and τ^* of Eq. 4.3, respectively. τ^* for each device is plotted against Ca as shown in Fig. 4.10a. The plot indicates that the increase of τ^* occurs in all rectangular cross-section devices but not in the circular cross-section device. Using a logarithmic scale, as shown in Fig. 4.10b, τ^* is approximately proportional to $\sqrt{Ca}$.

l_0 —estimated from the intercept of each curve l_D versus q in Fig. 4.8—also shows a strong increase against decreasing Ca in devices with rectangular cross-section (see Fig. 4.11a). Similar to τ^* , l_0 seems not to correlate to Ca in the device with the circular cross-section. As an initial guess, l_0 is assumed to be proportional to Ca^{-1} and follows the function $l_0 = \frac{\phi}{Ca} + \lambda_0$, with ϕ and λ_0 as constants independent of Ca . ϕ and λ_0 may depend on geometrical factors such as

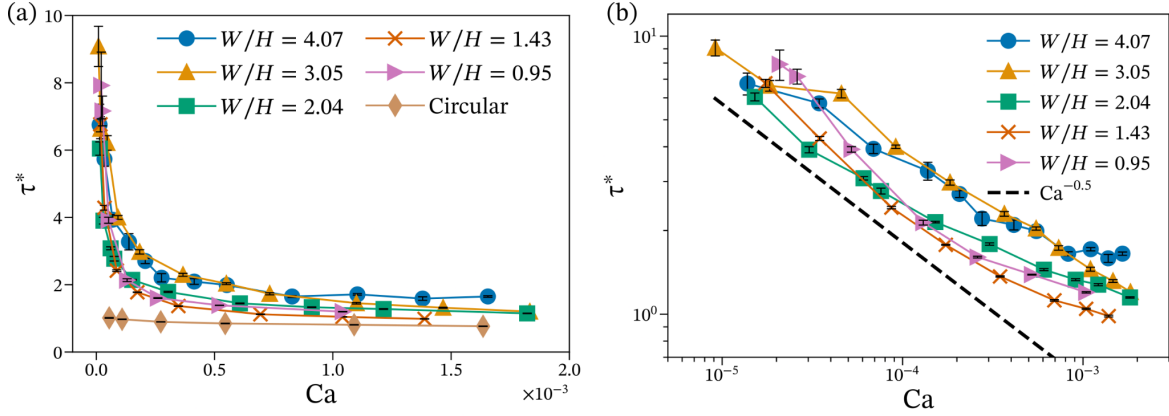


Fig. 4.10: τ^* versus Ca plotted in (a) linear scale and (b) logarithmic scale. τ^* were obtained from the slope of the linear fitting lines in Fig. 4.8. In (a) τ^* resulting from the cross-junction with rectangular cross-section increases greatly compared to the one resulting from the circular cross-section device. In (b) the measured τ^* is compared with the line $Ca^{-0.5}$.

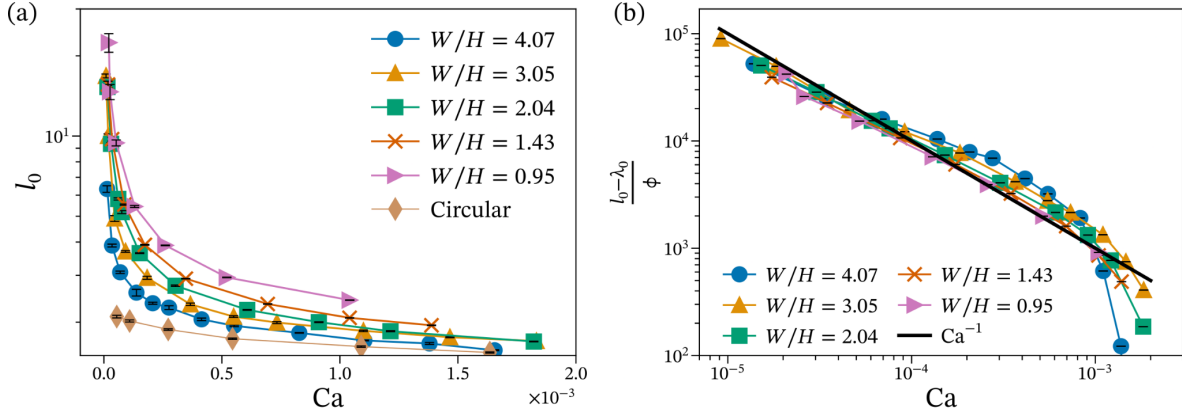


Fig. 4.11: The increase l_0 against Ca . (a) l_0 increases greatly in the rectangular cross-section junction. l_0 were obtained from the intercept of the linear relation of l_D versus q in 4.8a-f. (b) The master curve Ca^{-1} fits very well to the data $(l_0 - \lambda_0)/\phi$.

W/H . The coefficients were determined by curve fitting to the data l_0 versus Ca for each device separately, using the fit function $l_0 = \frac{\phi}{Ca} + \lambda_0$. Using the fitting values for the different devices, the expression $\frac{l_0 - \lambda_0}{\phi}$ was evaluated and plotted against Ca . The result, as shown in Fig. 4.11b, is a master plot of $\frac{l_0 - \lambda_0}{\phi}$ versus Ca which shows that all data series are approximately collapsed onto the line Ca^{-1} .

Observation of the neck shape before pinch-off also confirms the Ca -dependency of the droplet formation. Fig. 4.12a shows the elongation of the neck with decreasing Ca in the device with $W/H = 4.07$ compared with the shape of the neck from the device with a circular cross-section. Measurement of the area under and above the neck before pinch-off Θ shows an increase against decreasing Ca . Additionally, it is possible to obtain τ directly from observing the experimental videos recorded at the high frame rate. τ^* – dimensionless necking time – is then calculated by $\tau^* = \tau \cdot Q_C / HW^2$. An example of such a measurement result is shown in Fig. 4.12b. Interestingly, the direct measurement of τ^* also shows dependency to $\sim Ca^{-0.5}$, in agreement with the previous type of measurement as shown Fig. 4.12b.

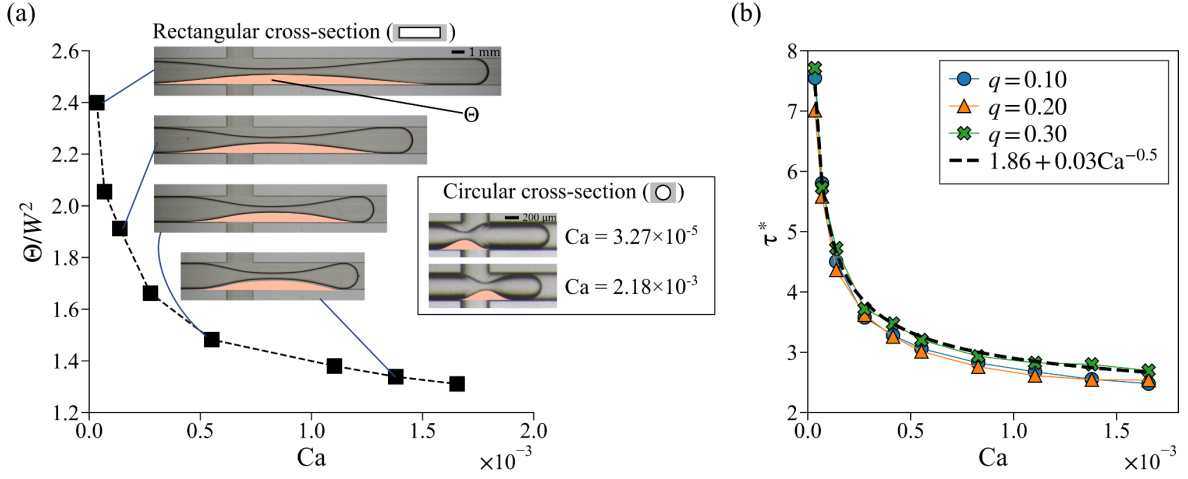


Fig. 4.12: (a) The elongation of the neck at very low Ca in the rectangular and circular cross-section junction. (b) τ^* measured from experiment with various Ca and q . The dashed-black line is the curve $a + bCa^{-0.5}$ with a and b as the curve fitting parameters.

4.1.4 Neck evolution details: the self-similar evolution and filamenting.

To understand the nature of droplet formation at very low Ca , it is important to understand the dependency of τ^* and l_0 to the flow parameters. Thus, it is important to observe the detail of the necking process, which was done by another experimental measurement with higher space and time resolution. The details of the experiment procedure and the measurement method have been described previously in chapter 3, and one example of such measurement is shown in Fig. 4.13. Fig. 4.13a shows an image of a droplet during the necking stage. The colorful

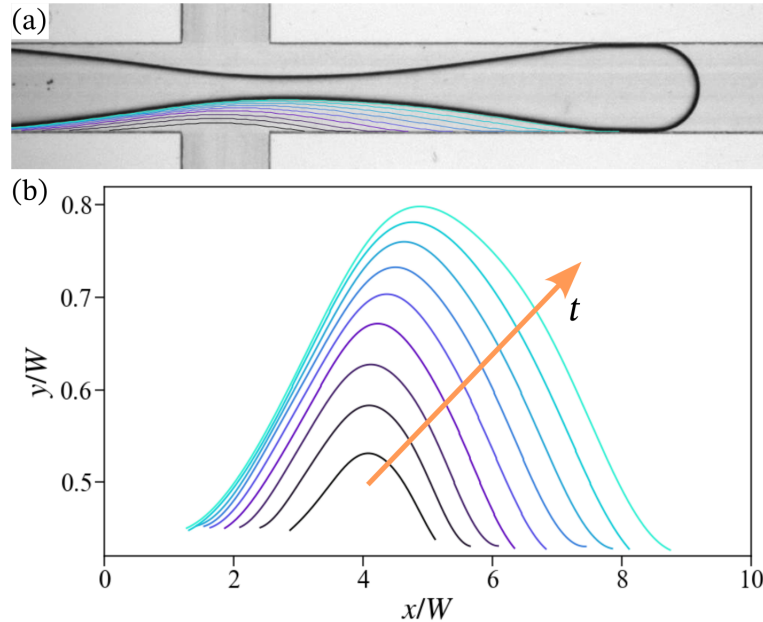


Fig. 4.13: (a) The neck outline is taken from a constant time interval (3.33 s) and overlaid on top of the final image. The brightness gradient of the lines shows the increasing time (b) Neck outline evolution. The color of the line corresponds to the identical outline in (a). The outlines show a self-similar shape evolution with gradual movement in the direction of the main flow. Measurement were done from cross-junction with $W/H = 4.07$ and with flow condition $Q_C = 1.0$ ml/h, $Q_D = 0.2$ ml/h recorded at 30 fps.

lines are the outline of the neck, with the time interval between each line being 3.33 seconds. The outline evolution starts from the lowest line—the black line— and continues upward. The increasing brightness level of the outline shows the advancing time order. In Fig. 4.13b, the same outlines are plotted on a different scale of the coordinate system, showing the evolution more clearly. The measurement shows that the outline shape is almost identical over time, suggesting a self-similar evolution. It also indicates that the outline is slowly propagating downstream over time, shown by the closer and further distance between the lines on the left side and right side of the neck, respectively. The self-similar evolution is a useful property of the process, as the problem can be simplified by measuring a few characteristic parameters instead of analyzing the complete outline. For that, two parameters were chosen:

- The amplitude of the neck outline, denoted as A or a in dimensionless form, with $a = A/W$.
- The maximum width of the neck outline, denoted as Ω or ω in dimensionless form, with $\omega = \Omega/W$.

The measurement of a and ω in various Ca and q is shown in Fig. 4.14. The almost linear growth of a versus ω confirms the self-similarity of the neck evolution. However, the self-similarity breaks near the pinch-off ($a \gtrsim 0.26$). Above this line, a grows slower than ω until the pinch-off occurs. Here, the evolution where the neck seems to elongate without getting thinner is called *filamenting* process. Based on this observation, the necking process can be divided into two subprocesses: the self-similar evolution and the filamenting. The self-similar evolution is the dominant process in the necking because it is the majority of the necking process. The filamenting occurs in a shorter period within the necking process. Overall, the necking becomes longer and takes longer time with the decreasing Ca , in accordance with the measurement of droplet length as shown in Figs. 4.8 and 4.9. The insights obtained from these measurements are very crucial to this research, as they become the base for the mathematical model development.

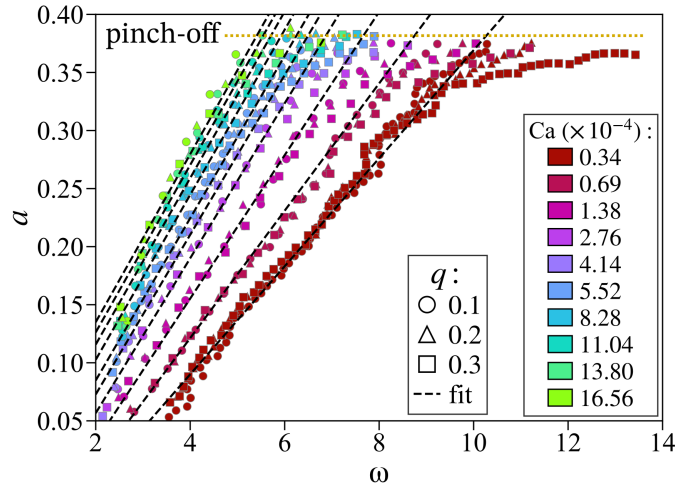


Fig. 4.14: Neck evolution shown as dimensionless amplitude a against dimensionless neck width ω for various q and Ca . The evolution of a against ω is approximately linear, confirming a self-similar growth. Near the end of the necking process, however, ω grows much more than a , breaking the self-similar growth. The dashed line shows the curve fitting line $a = \psi(\omega - \omega_0)$.

4.2 Mathematical model development

The presented experimental results have indicated that the droplet formation at very low Ca strongly depends on the flow rate ratio and the capillary number. To accommodate this finding,

a generalized equation of dimensionless droplet volume, Eq. 4.3, was proposed. However, the parameters l_0 and τ^* require more analysis. In this section, a semi-empirical mathematical model was developed to gain insights into the mechanisms that introduce the dependency of q and the Ca in the τ^* and l_0 .

4.2.1 Approximation of the planar neck profile

The complexity of the mathematical model was kept to a minimum, and it aimed to provide an approximate analytical solution to the problem without losing the essential understanding of the mechanism. Instead of solving the three-dimensional multiphase flow, the droplet outline seen from the top view is assumed to be two-dimensional curves (i.e., a flat droplet), neglecting variations in the channel depthwise direction. To be able to do that, the analysis was based on the experimental data obtained from the device with the highest aspect ratio ($W/H = 4.07$), i.e., the flattest device. At a later stage, the result of the flat-droplet analysis is verified by using experimental data from other devices with different aspect ratios. Additionally, the following analysis is restricted to the upper or lower half of the droplet, assuming interface symmetry relative to the main channel middle axis.

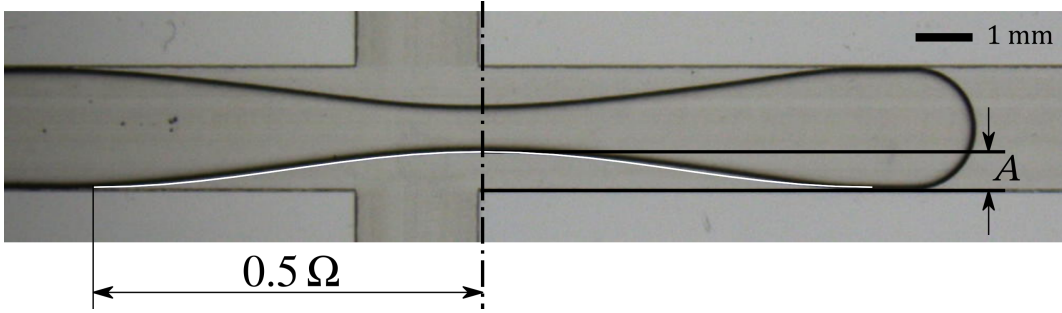


Fig. 4.15: The neck of a forming droplet, as seen from a top view image, takes the shape of part of a sine wave, with amplitude A and wavelength $\Omega/2$, as evident from the overlay of this shape (white line) on a prototypical image. The image was taken in the device with $W/H=4.07$, during necking with $Ca = 1.38 \times 10^{-4}$ and $q = 0.3$.

As seen from the top, the two-dimensional profile of the neck appears to take the shape of part of a sine wave (or cosine), with $A/2$ and Ω as the sine wave amplitude and wavelength, respectively. The neck profile is approximated with

$$h(x) = 0.5A \left[1 + \cos \left(\frac{2x}{\Omega} \pi \right) \right]. \quad (4.4)$$

Eq. 4.4 fits the neck profile very well, as seen from the overlaid white line in Fig. 4.15. The cosine function describes the neck profile during the self-similar evolution, where the ratio between A and Ω is approximately constant over a large portion of the necking stage. Using Eq. 4.4, the instantaneous volume area under the curve, V_N can be approximated from the integration, $V_N \approx \int_{-\Omega/2}^{\Omega/2} h(x) dx$, which can be written as:

$$V_N \approx H A \Omega. \quad (4.5)$$

In dimensionless form, the volume is normalized by HW^2 , resulting in $v_N = a\omega$. The curvature can be approximated by the curvature equation of a plane curve $y = h(x)$, $\kappa = h'' / [1 + (f')^2]^{3/2}$ which is commonly simplified to $\kappa = h''(x)$. The curvature reads:

$$\kappa(x) = -\frac{2A\pi^2}{\Omega^2} \cos \left(\frac{2x}{\Omega} \pi \right) \quad (4.6)$$

4.2.2 Necking equation of motion and its verification by using stop-flow experiment

The instantaneous interface shape of the neck and its evolution stems from a balance between viscous forces and interfacial forces. The order of magnitude of the viscous force follows from considering the flow of the CP in the gap between the interface and the wall. The interface deformation due to CP flow is described using two orthogonal directions of the flow: along the main channel (changing Ω) and transverse to it (changing A). The flow velocities in both directions can be approximated by $\frac{d\Omega}{dt}$ and $\frac{dA}{dt}$, respectively. Furthermore, viscous forces associated with the motion in the principal directions can be estimated as $\frac{\mu_C}{\Lambda} \frac{d\Omega}{dt}$ and $\frac{\mu_C}{\Xi} \frac{dA}{dt}$, respectively, where Λ and Ξ are the coefficients of the length dimension characterizing the geometry-dependent shear magnitude in the two directions.

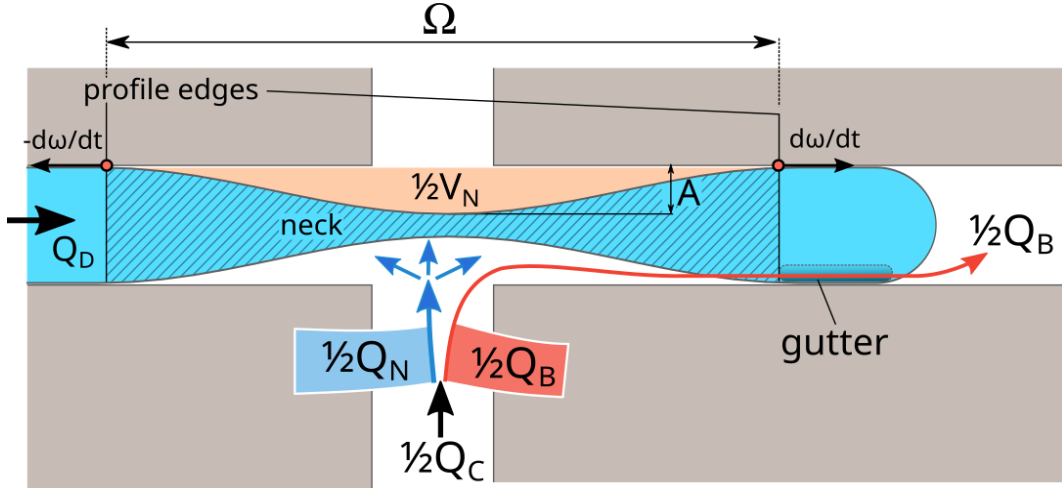


Fig. 4.16: The schematic of the necking process.

Assuming that the out-of-plane interface shape is imposed by the spacing between the top and bottom walls along the entire neck, the interface can only adjust its shape in the in-plane direction visible in the top view images (see Fig. 4.15). The order of magnitude of the interfacial force associated with the in-plane adjustment follows directly from the interface curvature, Eq. 4.6, where the curvature scales with the ratio $\sim \frac{A}{\Omega^2}$.

The net positive flow that deforms the neck simultaneously increases A and Ω over time (see Fig. 4.16). Based on the self-similarity evolution of the interface, the growth of the neck width Ω can be assumed as proportional to the growth of the neck amplitude A . Furthermore, the viscous forces are always balanced by the interfacial forces associated with the instantaneous shape of the neck. The instantaneous curvature, Eq. 4.6, is always in favor of the motion of the surface along the main channel (increasing Ω) while opposing the motion in orthogonal to the main channel, i.e., opposing the motion of A . Taking $\frac{A}{\Omega^2}$ as a representative value for the order of magnitude of the curvature, the pressure difference along the interface stemming from interface forces is proportional to $\gamma \frac{A}{\Omega^2}$. With this consideration, the equation of motion associated with the flow in two principal directions can be approximated as:

$$\frac{\mu_C}{\Xi} \frac{d\Omega}{dt} = \frac{\mu_C}{\Lambda} \frac{dA}{dt} + \gamma \frac{A}{\Omega^2}. \quad (4.7)$$

To describe how the interface evolves, i.e., how A and Ω simultaneously evolve, it is required to obtain the solution of Eq. 4.7. However, as there are two unknown variables in Eq. 4.7, it is not possible to solve it analytically. A and Ω are related to each other by the conservation of mass, Eq. 4.5. However, the instantaneous volume under the neck, V_N is unknown because the flow rate which the neck is filled with, Q_N is unknown. This leaves Eq. 4.7 analytically unsolvable unless there are additional equations.

However, it is also of interest to check if Eq. 4.7 is valid at this point. To check this validity, a special experiment called the stop-flow experiment was performed (for details, see subsection 3.5.6). In short, the experiments were performed as follows. First, the whole outlet channel was filled with DP, and the DP flow was stopped. Second, the CP was injected at a fixed flow rate to squeeze the DP in the junction, allowing the necking process to be similar to the droplet-formation process. Third, the CP flow was stopped before the neck pinch-off (Fig. 4.17a). During relaxation, the evolution of Ω and a is measured as shown in the points in Fig. 4.17b. The two cases differ by the flow rate from which the initial neck shapes were obtained. The flow rates of the CP of Cases I and II are 3 ml/h and 4 ml/h, respectively.

Given that after stopping the flow, the resistance to flow through the gutters is significantly larger than the resistance to flow in the neck region, it is expected that the CP volume accumulated behind the neck will remain constant during relaxation. Hence, there is no flow of the CP into/out of the neck region, i.e., $V_N = A_0 \Omega_0 H = \text{constant}$, with A_0 and Ω_0 as the amplitude and the width of the neck at time zero. The time zero was taken as the time when the CP flow stopped.

With the mentioned assumption, a solution from Eq. 4.7 can be obtained. By performing substitution $A = A_0 \Omega_0 H$, the Eq. 4.7 can be simplified into $\frac{d\Omega}{dt} = \frac{\gamma A_0 \Omega_0}{\mu_C (\frac{1}{\Xi} \Omega^3 + \frac{1}{\Lambda} A_0 \Omega_0 \Omega)}$. Then, the closed form solution of Eq. 4.7 can be written as:

$$\Omega(t) = \Omega_0 \sqrt{-\frac{A_0 \Xi}{\Omega_0 \Lambda} + \sqrt{\left(\frac{A_0 \Xi}{\Omega_0 \Lambda} + 1\right)^2 + 4t \frac{\Xi A_0 \gamma}{\Lambda_0^3 \mu_C}}} \quad (4.8)$$

By using a set of dimensionless numbers: $\xi = \Xi/W$, $a = A/W$, $\omega = \Omega/W$ and $\tilde{\tau} = t \frac{\gamma}{\mu_C W}$ the solution can be written in dimensionless form as follows:

$$\omega(\tilde{\tau}) = \omega_0 \sqrt{\sqrt{\left(\frac{a_0 \xi}{\omega_0 \lambda} + 1\right)^2 + 4\xi \frac{a_0}{\omega_0^3} \tilde{\tau}} - \frac{a_0 \xi}{\omega_0 \lambda}}. \quad (4.9)$$

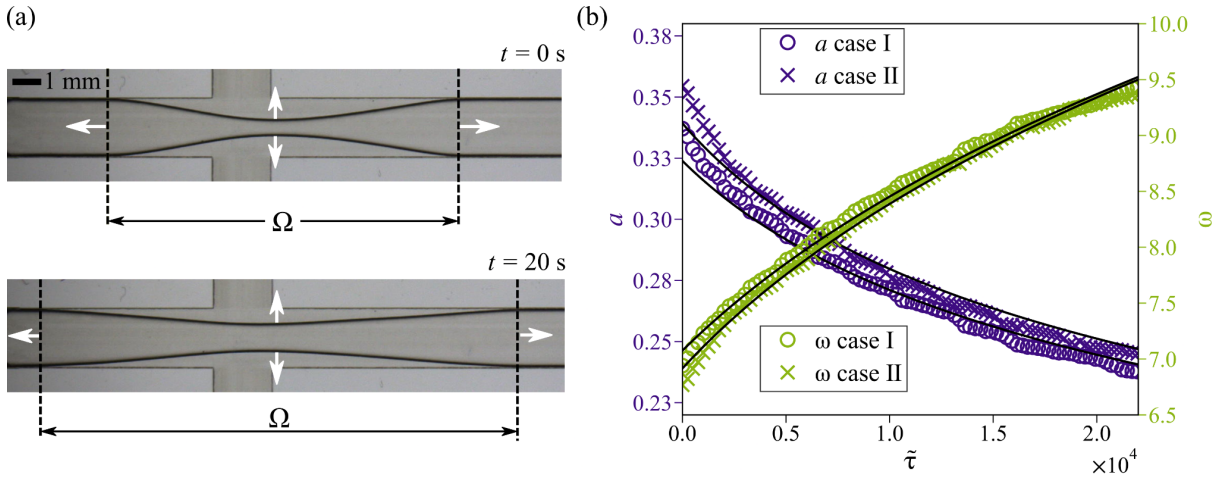


Fig. 4.17: (a) Relaxation of the interface after the CP flow was stopped ($t = 0$ s). (b) The experimental measurement points were compared to the solution provided in the Eq. 4.9 (solid black lines).

The curve fitting based on the stop-flow solution, Eq. 4.9, is shown as solid lines in Fig. 4.17b, describing the evolution of ω and a during the relaxation process very well. a is calculated by $a = \frac{a_0 \omega_0}{\omega}$ where ω is obtained from Eq. 4.9.

4.2.3 Leaking flow analysis

A droplet moving in a rectangular cross-section channel is understood to act like a leaking piston [61]. This is due to the possibility of the CP flowing through the unoccupied space in the corner of the channel, the so-called gutter. The seemingly simple problem of a droplet moving through a straight rectangular cross-section channel becomes quite complex [74] (or see the discussion of this matter in Section 2.1.2.6) since the relationship between droplet velocity and driving pressure drop is non-trivial problem [106]. It is still an active area of research, which commonly utilizes advanced numerical methods [107] due to the complexity of the problem. The same leaking flow through the gutter has been shown to be present and taken into play in the droplet formation in the microfluidic T-junction [60, 63]. Korczyk et al. [60] shows that the leaking flow in T-junction causes the extreme increase of l_D at vanishing Ca .

The objective of this subchapter is to investigate the leaking flow in the cross-junction device. At first guess, the leaking flow in cross-junction should not be much different from the one in T-junction. However, as will be shown later, there are some fundamental differences between them. Fig. 4.18a illustrates the flow condition during the necking process in a cross-junction. The CP flowing through the junction is divided into two types of flow: the necking flow rate Q_N that fills the volume behind the neck, V_N (see yellow arrow and area) and the leaking flow through the gutter, Q_G (see green arrow and area).

The leaking flow is analyzed by using an RC electrical circuit analogy shown previously in [60]. The analogous RC circuit of the fluid flow problem is shown in Fig. 4.18b. The method significantly simplifies the problem, as the three-dimensional fluid flow problem with its complex flow field is replaced by solving a linear DC circuit based on the balance of the pressure. To understand the circuit, first consider the conditions shown in the smaller Fig. 4.18c, in which CP flows through the junction with minimum interference of DP. The pressure drop across the junction ($P_0 - P_i$) is approximately in the order of magnitude of the viscous pressure drop,

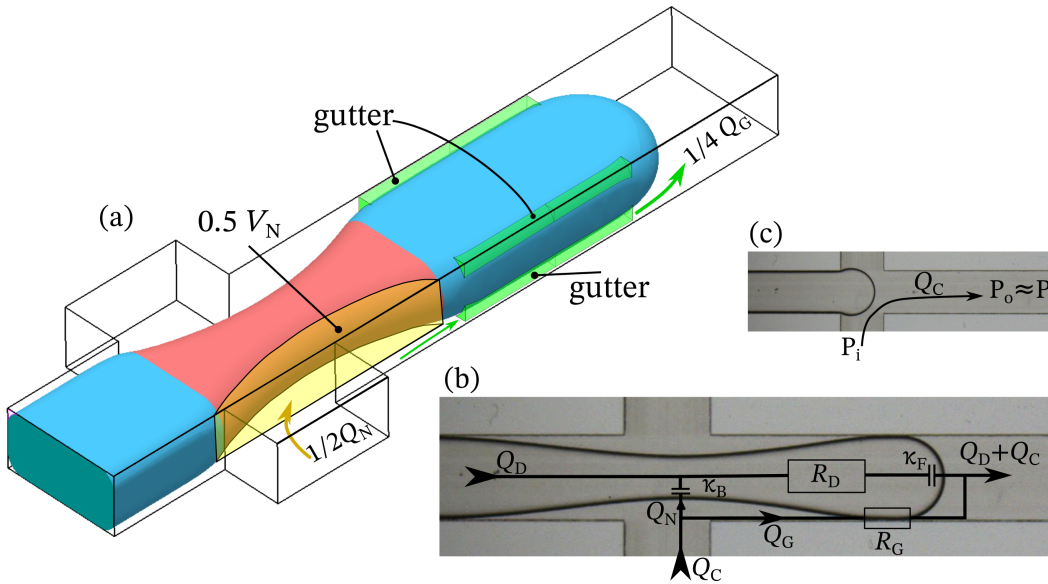


Fig. 4.18: (a) A 3D sketch of a droplet under necking in a cross-junction device. The red color marks the approximate neck. The yellow arrow shows the necking flow rates, Q_N , which supply the CP to the space on the left and right side of the neck (shown only one side as the yellow colored volume). The green region and arrow show the gutter and the gutter flow Q_G , respectively. (b) The schematic of the electrical DC circuit. The arrows represent the flow rate. The resistors (squares) and capacitors represent hydraulic resistance and Laplace pressure drop. The static pressure drop across the junction ($P_i - P_o$) is constant (c) During the filling stage, the static pressure drop is almost negligible.

$\propto \mu_C Q_C L / A_{cs}^2$ (Eq. 2.5), where L is the travel distance and A_{cs} is the cross-section area. This pressure drop is negligible $P_0 \approx P_i$, given a very short $L \approx W$. In the presence of the DP occupying the junction, the CP flow is divided into two, one part flowing into the neck region and the other part flowing through the gutters. The dividing flow is modeled as the branching of the electrical circuit line, as shown in Fig. 4.18.

Similar to above, the pressure drop across the flow through the gutter, ΔP_G is proportional to the viscous pressure drop $\mu_C Q_C L / A_G^2$; however, it is not negligible as the cross-section area is very small and the length varies by time. There are four gutters, each with hydraulic resistance denoted as R_G . In the electrical circuit, the four gutters are in parallel to each other, rendering the total resistance as $R_G/4$. The gutter cross-section area, A_G , is assumed to be constant along the gutter for simplicity. The gutter length is assumed to have an initial length, L_0 , that increases at the rate of Q_D/HW . The bypassing flow through the four gutters is denoted as $Q_B = Q_C - Q_N$. The pressure drop across the four gutters can be written as:

$$\Delta P_G = Q_B \cdot \frac{R_G}{4} = (Q_C - Q_N) \cdot \frac{\alpha \mu_C \left(L_0 + \frac{Q_D}{HW} t \right)}{4A_G^2}, \quad (4.10)$$

where α is the dimensionless correction factor [68].

On the other branch of flow, there are two factors contributing to the pressure drop. First, the viscous pressure drop of the DP flow, which is proportional to $(Q_D + Q_N) \cdot R_D$, where R_D is proportional to $\sim L_D/A_{cs}^2$. L_D is in the order of magnitude with gutter length, whereas $A_{cs} \approx WH$ is much larger compared to A_G , such that viscous pressure drop associated with DP flow can be safely neglected. Then the pressure drop across the droplet is mainly due to the Laplace pressure drop (Eq. 2.7), $\Delta P_L = \gamma (\kappa_{front} - \kappa_{neck})$, where $(\kappa_{front} - \kappa_{neck})$ is the curvature difference between the front of the droplet and the neck. The curvature of the droplet's front can be obtained from the principal radii at the front-center of the droplet: $0.5H$ and $0.5W$, renders $\kappa_{front} = \frac{2}{H} + \frac{2}{W}$. At the center of the neck, the principal radius in one direction is $0.5H$ while the curvature in the other direction has been described previously in Eq. 4.6 where at $x = 0$ the curvature is equal to $-2A\pi^2/\Omega^2$. The neck curvature reads as $\kappa_{neck} = \frac{2}{H} - \frac{2A\pi^2}{\Omega^2}$. Hence, the pressure drop across the droplet can be written as:

$$\Delta P_L = \gamma \left(\frac{2}{W} - \frac{2A\pi^2}{\Omega^2} \right) \quad (4.11)$$

The pressure drop between the inlet and the outlet is constant such that $\Delta P_G = \Delta P_L$, yields:

$$(Q_C - Q_N) \cdot \frac{\alpha \mu_C \left(L_0 + \frac{Q_D}{HW} t \right)}{4A_G^2} = \gamma \left(\frac{2}{W} - \frac{2A\pi^2}{\Omega^2} \right) \quad (4.12)$$

Recall the instantaneous necking flow rate ratio, $q_N = Q_N/Q_C$, and dimensionless time, $t^* = tQ_C/HW^2$, dimensionless form of Eq. 4.12 can be written as

$$\alpha (1 - q_N) \text{Ca} \frac{HW^3}{4A_G^2} (l_0 + qt^*) = 2 + 2\pi^2 \frac{a}{\omega^2}. \quad (4.13)$$

Solving for q_N yields

$$q_N = 1 - \frac{8A_G^2 (1 + \pi^2 \frac{a}{\omega^2})}{\alpha HW^3 \text{Ca} (l_0 + qt^*)} \quad (4.14)$$

Eq. 4.14 shows dependency q_N to Ca , and it is also decreasing by time. However, Eq. 4.14 also a function of time-dependent $\frac{a}{\omega^2}$. Considering $a < 1$ while $\omega \gg 1$, the ratio $\frac{a}{\omega^2}(\tau^*)$ is a decreasing quantity over time with order much less than unity. Therefore, for simplicity, the term $\frac{8A_G^2}{\alpha HW^3} (1 + \pi^2 \frac{a}{\omega^2})$ is replaced by its time-averaged value, κ . The equation for q_N becomes

$$q_N = 1 - \frac{\kappa}{\text{Ca} (l_0 + qt^*)} \quad (4.15)$$

Eq. 4.15 shows that the leaking mechanism in cross-junctions is considerably different from that in T-junctions. Although in both geometries, the flow around the forming droplet through the gutters depends on fluid properties and flow conditions, this dependency manifests in different ways. While in cross junctions, the flow around the forming droplet through the gutters decays over time, in T-junctions, it is time-constant [60].

Eq. 4.15 requires experimental verification; however, it is more straightforward to verify the resulting dimensionless necking volume, $V_N(t^*)$ because the neck area is directly observable in the experiments. Integrating of Eq. 4.15 over time, $v_N = \int_0^{t^*} q_N dt^*$, and multiply it with q , yields:

$$qv_N = qt^* - \frac{\kappa}{Ca} \ln \left(\frac{qt^*}{l_0} + 1 \right) \quad (4.16)$$

To verify Eq. 4.16, measurements were done for the area under the neck, θ , during the formation of droplets for various Ca and q values in the device with aspect ratio $W/H = 4.07$ using a higher image resolution and frame rate (see subsection 3.5.5 for details). The resulting curves (qv_N) are then fitted with Eq. 4.16, with qt^* as the independent variable and κ and l_0 the curve-fitting parameters. As shown in Fig. 4.19a, all measurements are well described by Eq. 4.16, with a constant value of $\kappa = 2.8 \times 10^{-5}$, while the values of l_0 are Ca dependent. The values of l_0 are inversely proportional to Ca , as shown Fig. 4.19b. Additionally, curve fitting of function $l_0 = \frac{\kappa_0}{Ca}$ fits very well the values of l_0 with $\kappa_0 = 4.5 \times 10^{-5}$ as a curve fitting parameter.

After Eq. 4.16 is confirmed by experimental measurement. The focus now is to return to Eq. 4.15 since the obtained variables immediately provide insight into the fraction of the incoming CP contributing to necking. A characteristic dimensionless time of leaking τ_{HL}^* is defined as the time when the flow around the forming droplet through the gutters drops to half of its maximal available value so that q_N reaches a value of 0.5, i.e., $\tau_{HL}^* = \frac{2\kappa - \kappa_0}{qCa}$. For all curves in Fig. 4.19a, τ_{HL}^* is considerably smaller than the duration of the entire necking. Thus, in further analysis, beyond the short initial period of necking, the Ca variation of q_N introduced

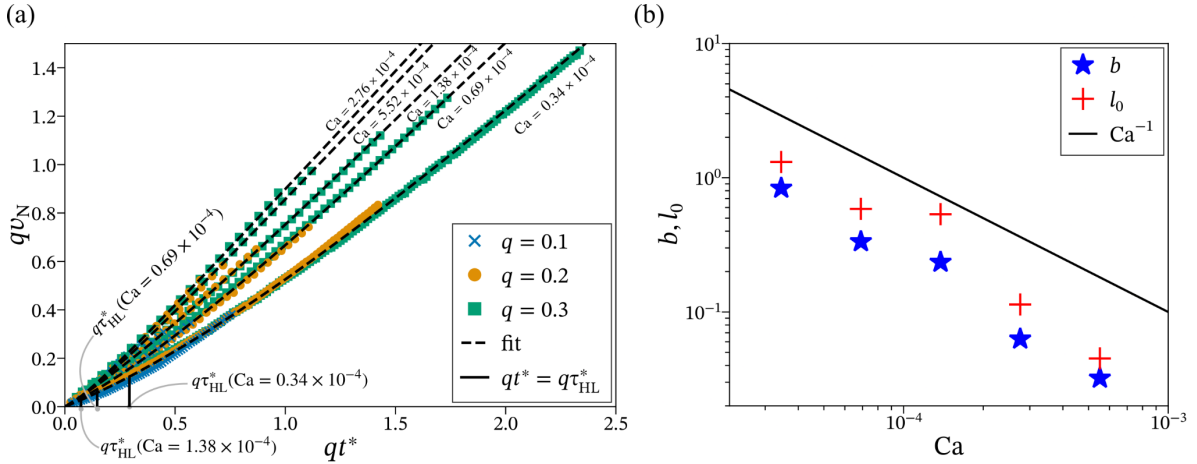


Fig. 4.19: Experimental analysis of the leaking flow. Values of v_N and τ^* were measured from the images recorded using appropriately high spatial and temporal resolutions. (a) qv_N plotted as a function of qt^* for different values of Ca and q . According to Eq. 4.16, different data series collapse on separate lines, each characterized by unique Ca . Dashed lines show the curve-fitting result of Eq. 4.16 with $b = \frac{\kappa}{Ca}$ and l_0 set as the curve-fitting parameters to the data series with the same Ca . Solid vertical lines show the three highest $q\tau_{HL}^*$ values for the three smallest values of Ca , as indicated by the attached labels. (b) Ca dependency of the fitted parameters b and l_0 . The parameters κ and κ_0 were estimated by fitting to experimental point functions $b = \frac{\kappa}{Ca}$ and $l_0 = \frac{\kappa_0}{Ca}$, respectively. The fittings provided the values $\kappa = 2.8 \times 10^{-5}$ and $\kappa_0 = 4.5 \times 10^{-5}$.

by the second term in Eq. 4.15 is considerably small, in which a simplified approximation, $q_N(t^* \gg \tau_{HL}^*) \approx q_N(\tau^*) \sim 1$ is assumed for following analysis.

4.2.4 Dependency of initial droplet length to Ca

Thus far, the discussion has been focused around the mechanisms that give rise to q and Ca dependency during the necking stage. Since the droplet shape before pinch-off is q and Ca dependent, it may be the same case for the filling stage and the droplet length at the end of the filling stage (l_0 in Eq. 4.4). Therefore, the droplet tip position relative to the junction before necking starts (i.e., shown as the image when $t = 0$ s in Fig. 4.2), denoted as L_{T0} , is measured over a wide range of q and Ca , as illustrated in Fig. 4.20. Although L_{T0} is not a direct measurement of l_0 , it is expected that they have similar Ca dependency. Fig. 4.20 shows $L_{T0} \propto Ca^{-1}$, which shows that the Ca dependency in the measured length of the droplets is introduced not only in the necking stage but also in the filling stage. The results also confirmed the proportionality $l_0 \propto Ca^{-1}$ obtained from the leaking flow analysis in the previous section.

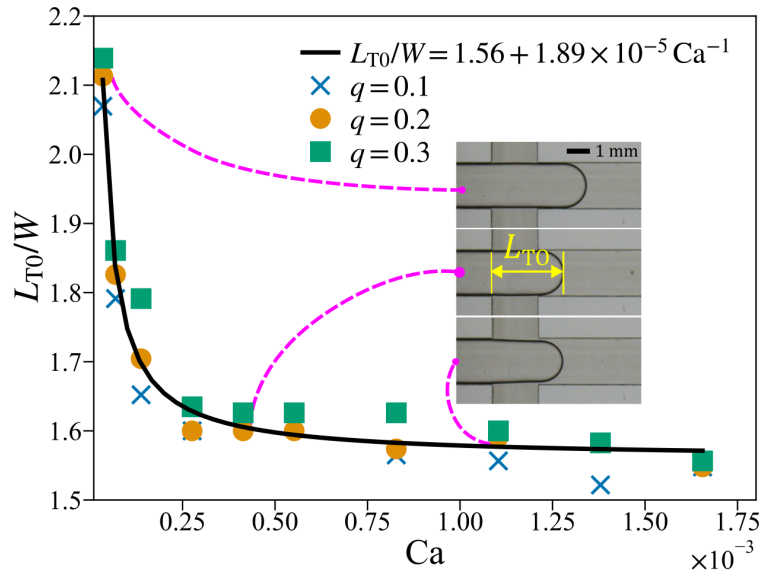


Fig. 4.20: Measurement of the droplet tip position (points) relative to the junction at the start of necking, L_{T0} , versus Ca shows the Ca dependency of $\sim Ca^{-1}$. The solid line is the curve fitting of L_{T0} as a function of Ca . The presented measurements are taken from experiments using the device with aspect ratio $W/H = 4.07$.

4.2.5 Approximating the necking duration based on the self-similar necking stage

As shown previously in Fig. 4.14, the necking process can be divided into two kinds of processes: self-similar evolution and filamenting. A threshold value to divide these two processes was chosen as $a = 0.26$.

For the lower regime ($a \leq 0.26$), the self-similar evolution follows the shape described previously in Eq. 4.4 such that the dimensionless volume under the neck shape takes $v_N = a\omega$. The instantaneous dimensionless necking flow rate is related to the neck volume by $q_N = \frac{d(a\omega)}{dt^*}$. Both a and ω are a function of time, such that the necking flow rate can be written as

$$q_N = \frac{d(a\omega)}{dt^*} = a \frac{d\omega}{dt^*} + \omega \frac{da}{dt^*}. \quad (4.17)$$

As shown in Fig. 4.14, a versus ω relation can be described by a linear equation $a = (\omega - \omega_0)\psi$, with ψ being the tangent of the term $\psi = \frac{da}{d\omega}$. Due to the self-similar process,

the tangent, ψ , also can be approximated as the ratio between the amplitude and the width of the neck, $\psi \approx \frac{a}{\omega}$, which is constant.

$$q_N = \frac{d(a\omega)}{dt^*} = \frac{d(\psi\omega^2)}{dt^*} = \psi \frac{d\omega^2}{dt^*} = \psi \frac{d\omega^2}{d\omega} \frac{d\omega}{dt^*} = 2\psi\omega \frac{d\omega}{dt^*} \quad (4.18)$$

The above equation requires $\frac{d\omega}{dt^*}$ which can be obtained from Eq. 4.7, rewritten here in dimensionless form:

$$\frac{1}{\xi} \frac{d\omega}{dt^*} = \frac{1}{\lambda} \frac{da}{dt^*} + \frac{a}{\omega^2} \text{Ca}^{-1}, \quad (4.19)$$

where $\xi = \Xi/W$ and $\lambda = \Lambda/W$ are the dimensionless form of the coefficient in Eq. 4.7. Substituting Eq. 4.18 into Eq. 4.19 and simplifying yields:

$$\frac{q_N}{2\psi} = \frac{\xi}{\lambda} \frac{q_N}{2} + \xi\psi\text{Ca}^{-1}. \quad (4.20)$$

Multiplying Eq. 4.20 by $\psi\text{Ca}/\xi$ gives:

$$0 = \psi^2 + \left(\frac{1}{2\lambda} q_N \text{Ca} \right) \psi - \frac{1}{2\xi} q_N \text{Ca}, \quad (4.21)$$

which can be solved for ψ , yielding:

$$\psi(\text{Ca}) = 2 \left(\frac{\xi}{\lambda} + \sqrt{\left(\frac{\xi}{\lambda} \right)^2 + \frac{8\xi}{q_N \text{Ca}}} \right)^{-1}. \quad (4.22)$$

ψ is the tangent of the self-similar necking process, which was assumed to be constant. However, it has time-dependency that comes from the function of $q_N(t^*)$ appears in Eq. 4.22. Substituting the relation for q_N (Eq. 4.15) to the above Eq. 4.22 gives:

$$\psi(\text{Ca}) = 2 \left(\frac{\xi}{\lambda} + \sqrt{\left(\frac{\xi}{\lambda} \right)^2 + \frac{8\xi}{\text{Ca} \left(1 - \frac{\kappa}{\kappa_0 + q\text{Cat}^*} \right)}} \right)^{-1}. \quad (4.23)$$

Eq. 4.23 is time-dependent due to the presence of the term $q\text{Cat}^*$. Here, $q\text{Ca}$ represents another form of the capillary number, defined as $Q_D\mu_C/(HW\gamma)$, which is a constant. Experimental measurements indicate that $\psi = \frac{a}{\omega}$ remains approximately constant throughout the self-similar growth, as shown in Fig. 4.14. This self-similar behavior suggests that the influence of $q\text{Cat}^*$ on ψ is negligible. Consequently, the term $(1 - \kappa/(\kappa_0 + q\text{Cat}^*))$ is assumed to be constant and, for simplicity, is incorporated into the constant ξ in Eq. 4.23 yields:

$$\psi(\text{Ca}) = 2 \left(\frac{\xi}{\lambda} + \sqrt{\left(\frac{\xi}{\lambda} \right)^2 + \frac{8\xi}{\text{Ca}}} \right)^{-1}. \quad (4.24)$$

It is challenging to obtain an explicit analytical solution for τ^* , and increasing the complexity of the current mathematical model does not yield more meaningful results. However, the semi-empirical relation in Eq. 4.24 can be used to approximate the dependence of τ^* on Ca , which is essential to this work and can be validated experimentally.

As shown in Eq. 4.4, the dimensionless necking time is given by the total volume under the neck divided by the average necking flow rate:

$$\tau^* = \frac{v_{N0}}{\langle q_N \rangle}.$$

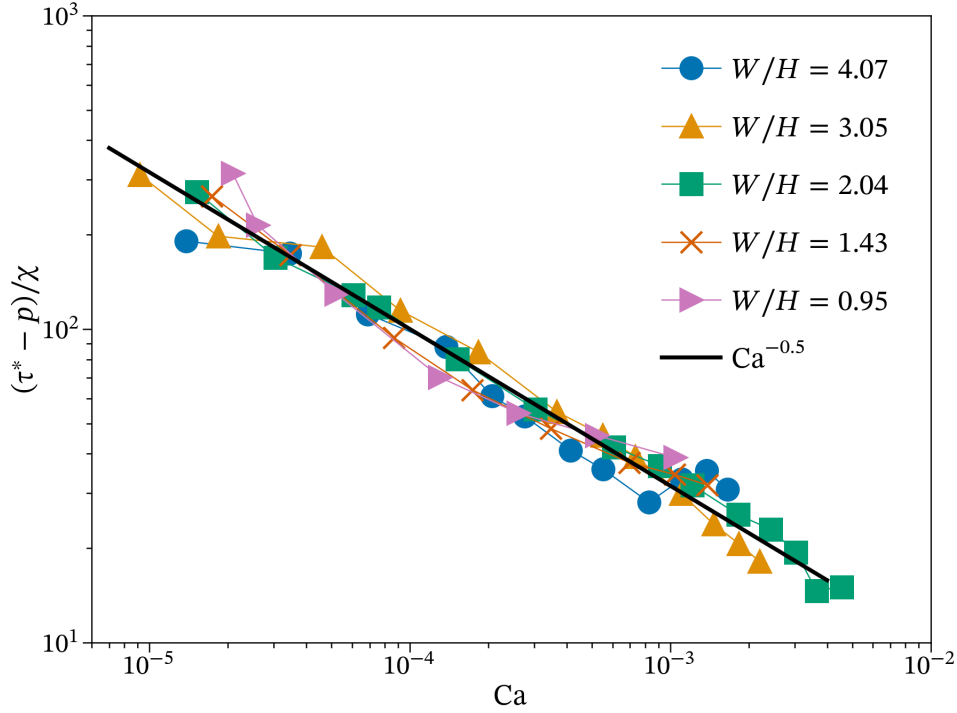


Fig. 4.21: Comparison between measurements (τ^* , points) and theoretical predictions (Eq. 4.26, solid lines) for devices with various W/H . The curve fitting results show good agreement between the experimental result and Eq. 4.26. The dimensionless fitting parameters of each device are: $W/H = 4.07$: $\chi = 2.86 \times 10^{-2}$, $p = 0.76$; $W/H = 3.05$: $\chi = 2.77 \times 10^{-2}$, $p = 0.63$; $W/H = 2.04$: $\chi = 1.92 \times 10^{-2}$, $p = 0.64$; $W/H = 1.43$: $\chi = 2.35 \times 10^{-2}$, $p = 0.24$; $W/H = 0.95$: $\chi = 2.84 \times 10^{-2}$, $p = 0.10$.

Additionally, the instantaneous dimensionless necking volume is related to Eq. 4.24 by $v_N = a^2/\psi$. At the end of the self-similar necking stage, a is taken as a constant, $a = 0.26$. The necking time resulting from the self-similar necking stage, τ_1^* , can be expressed as:

$$\tau_1^* = \frac{0.26^2}{\psi \langle q_N \rangle}.$$

The analysis of τ_{HL}^* in Section 4.2.3 indicates that q_N increases rapidly during the initial phase, eventually reaching its asymptotic value $q_N(\tau^*)$, which is a constant close to unity (see Fig. 4.19). Therefore, as the end of the self-similar necking stage approaches τ^* , the initial growth of q_N can be neglected. Consequently, the time-averaged dimensionless necking flow rate, $\langle q_N \rangle$, can be approximated as unity. Considering these arguments, $\tau_1^*(Ca)$ can be approximated as:

$$\tau_1^* \approx \frac{0.26^2}{2} \left(\frac{\xi}{\lambda} + \sqrt{\left(\frac{\xi}{\lambda} \right)^2 + \frac{8\xi}{Ca}} \right). \quad (4.25)$$

Eq. 4.25 shows that the dimensionless necking duration is proportional to the square root of the capillary number, approximately $\tau^* \propto \sqrt{Ca}$. Experimental measurements in Fig. 4.15 indicate that the self-similar necking stage dominates the process. Therefore, the dependence of the overall necking stage, τ^* , on Ca is approximated from Eq. 4.25. Finally, the scaling of τ^* with the capillary number is proposed as:

$$\tau^* = \frac{\chi}{\sqrt{Ca}} + p, \quad (4.26)$$

where χ and p are constant parameters that may depend on the device geometry. The experimental results shown in Fig. 4.12b demonstrate that Eq. 4.26 provides an excellent fit to the direct measurements of τ^* obtained from high-frame-rate experiments.

To validate the proposed relation, measurements from all rectangular cross-section devices presented earlier in Fig. 4.10 were compared with Eq. 4.26. Curve fitting was performed separately for each device, yielding the parameters χ and p for each geometry. Subsequently, the experimentally obtained values of τ^* for each device were plotted as $(\tau^* - p)/\chi$. According to Eq. 4.26, the quantity $(\tau^* - p)/\chi$ for all devices should collapse onto the same curve, proportional to $\text{Ca}^{-0.5}$. The results show strong agreement between the proposed scaling in Eq. 4.26 and the experimental data, as illustrated in Fig. 4.21.

4.2.6 Filamenting process

Previously, it has been shown that the necking of the lower regime can be estimated as a self-similar evolution of the cosine shape, in which the assumption of constant a/ω is relevant. A short period of neck elongation in the streamwise direction follows after this self-similar evolution. In this mode, the cosine shape breaks due to its apex flattening as the thinnest part of the neck elongates. As shown in Fig. 4.14 for $a > 0.26$, ω grows substantially higher compared to the growth of a . As a increases slowly, eventually, it reaches a critical value where the filament breaks, ending the droplet formation process. This thin-long neck, called as the filament, is shown schematically in Fig. 4.22(a).

Assuming the filament is a box with a dimensionless length ε and width δ (see Fig. 4.22a), the dimensionless volume of the filament is given by the product $\varepsilon\delta$, denoted as $\rho = \varepsilon\delta$. Note that the filament has a real height equal to the height of the channel, which corresponds to unity in the dimensionless form. The volume ρ evolves due to the inflow of DP; therefore, its increment $\Delta\rho = \rho - \rho_0$, where ρ_0 is the initial volume for $a = 0.26$, is proportional to the product of q and the time increment: $\Delta\rho \approx q\Delta t^*$. Assuming that ε grows linearly with time, such that $\Delta\varepsilon \propto \Delta t^*$, the volume ρ can be expressed as $\rho = cq\Delta\varepsilon + \rho_0$. Consequently, the equation for δ is given by:

$$\delta = \frac{\rho_0 + cq\Delta\varepsilon}{\varepsilon}, \quad (4.27)$$

where c is a constant.

Next, it is assumed that ε elongates at the same rate as ω , such that $\Delta\varepsilon \approx \Delta\omega$, where $\Delta\varepsilon = \varepsilon - \varepsilon_0$, and ε_0 is the initial length at the start of the filamenting process. The parameter δ is related to the amplitude of the neck shape, a , by the equation:

$$a = 0.5(1 - \delta).$$

Thus, the relationship between a and the increment of the filament width, $\Delta\omega$, is:

$$a = \frac{1}{2} \left(1 - \frac{\rho_0 + cq\Delta\omega}{\Delta\omega + \varepsilon_0} \right). \quad (4.28)$$

The fitting result of Eq. 4.28 to the experimental data for $a > 0.26$ is shown in Fig. 4.22b. From the curve fitting, the following relation was obtained:

$$a = \frac{1}{2} \left(1 - \frac{0.48 + 0.48q\Delta\omega}{\Delta\omega + \varepsilon_0} \right), \quad (4.29)$$

where $\varepsilon_0 = 1.32 + 2.51 \times 10^{-4}(1.63 \times 10^{-4} + \text{Ca})^{-1}$. Fig. 4.22 demonstrates good agreement between the developed model and the experimental results.

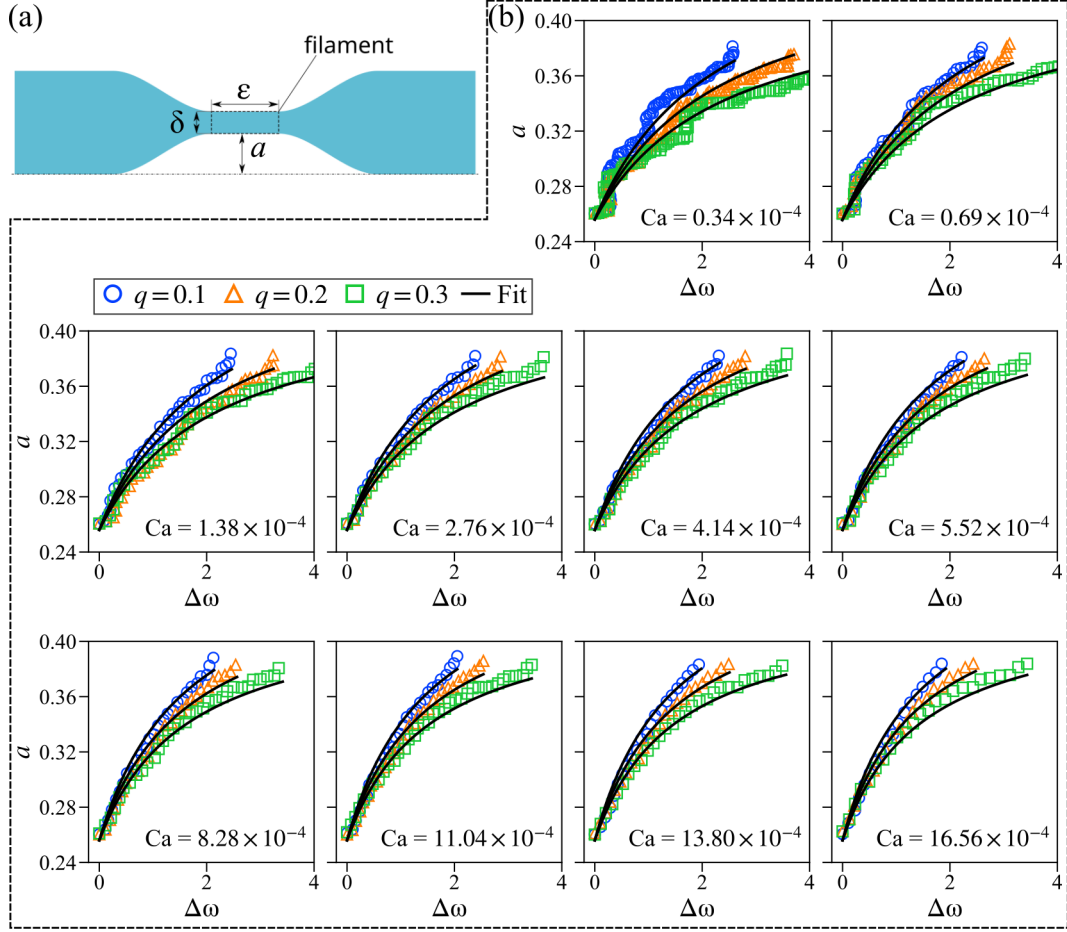


Fig. 4.22: (a) In the upper regime $a > 0.26$, the increase in filament length ε is faster than the thinning of filament width δ . (b) Kinematics of the filamenting stage. Points are the experimental data for different values of q . The solid lines are the fitting results of Eq. 4.28 with fitting parameters ρ_0 , c and $\varepsilon_0 = 1.32 + 2.51 \times 10^{-4}(1.63 \times 10^{-4} + Ca)^{-1}$. Graphs without a y-axis label and axis numbers have their y-axis shared with the figure on their left. Measurements were taken in the device with $W/H = 4.07$.

4.2.7 Generalized squeezing scaling law in cross-junction device

Thus far, the mathematical development has been aimed at gaining insight into the parameters l_0 and τ^* of the generalized scaling law (Eq. 4.3), rewritten here as $l_D = l_0 + \tau^*q$. The analysis in the preceding sections demonstrates that both l_0 and τ^* depend on the capillary number, Ca . Specifically, l_0 is influenced by Ca due to the leakage effect, with a proportionality of Ca^{-1} . The relationship $l_0 = \lambda_0 + \frac{\phi}{Ca}$ has been shown to fit the experimental measurements very well, as illustrated in Fig. 4.11b and Fig. 4.20.

The necking duration increases as Ca decreases, primarily due to the elongation of the neck. This elongation results from the deformation of the interface, a natural relaxation process driven by surface tension. Such elongation is feasible in microfluidic cross-junctions because these geometries do not impose constraints that would interfere with DP motion in the main channel direction. The elongation reduces the necking rate, and the slower necking process allows more time for further elongation, forming a self-reinforcing cycle. Consequently, necking in microfluidic cross-junctions at very low Ca becomes significantly more complex than in T-junctions, as reflected by the more intricate mathematical model.

However, a simplified scaling law for τ^* as a function of Ca was proposed (Eq. 4.26), which has been shown to provide a good fit to the experimental measurements, as demonstrated

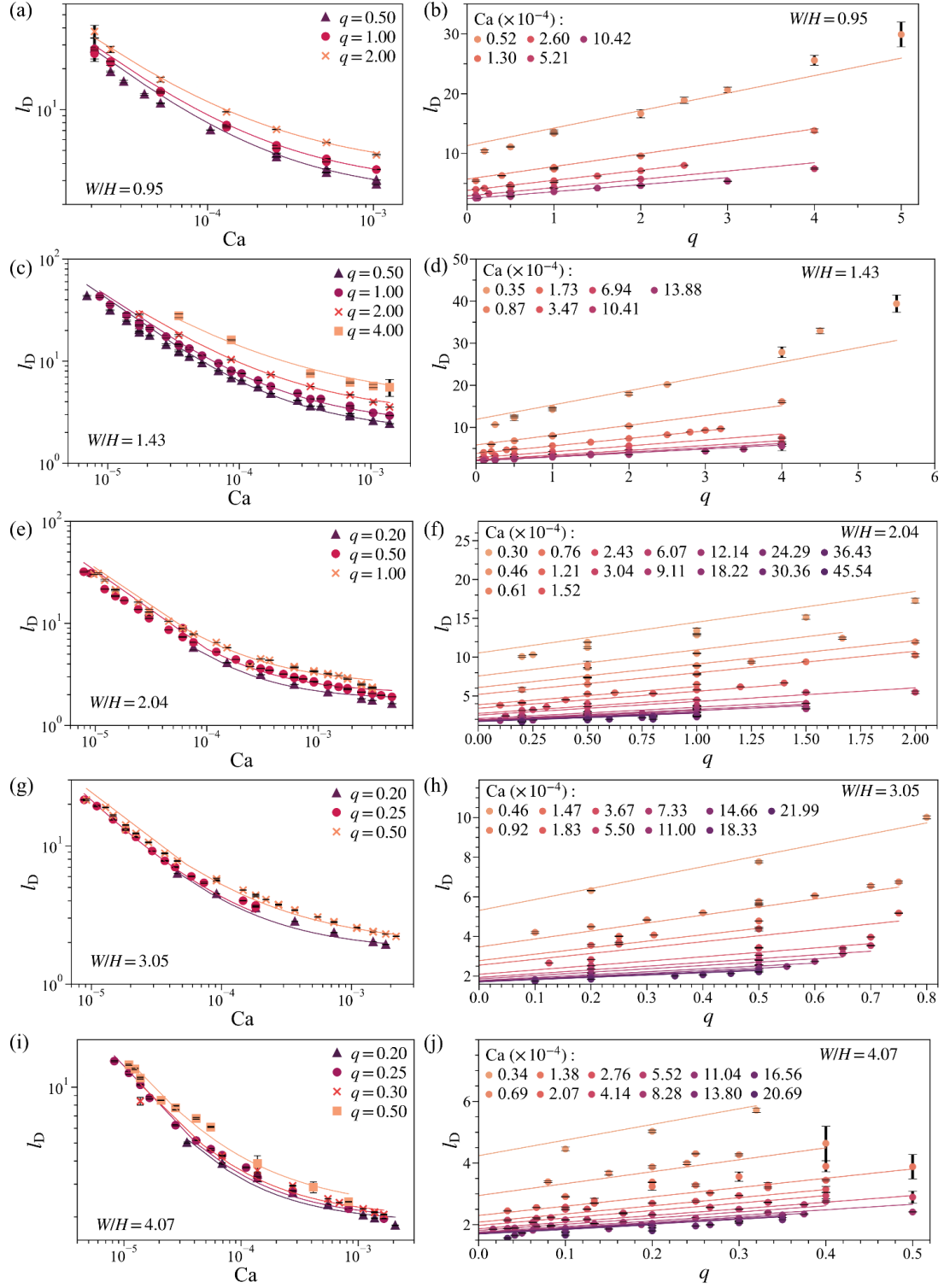


Fig. 4.23: Comparison between measurements (points) and theoretical predictions (Eq. 4.30, solid lines) for devices with W/H : (a-b) 0.95; (c-d) 1.43 (e-f) 2.04 (g-h) 3.05 (i-j) 4.07.

in Fig. 4.21 and Fig. 4.12b. Incorporating the observed Ca -dependencies of l_0 and τ^* , the generalized squeezing law for cross-junctions is given by:

$$l_D = \lambda_0 + \frac{\phi}{Ca} + q \left(\frac{\chi}{\sqrt{Ca}} + p \right), \quad (4.30)$$

where the parameters λ_0 , χ , p , and ϕ are independent of Ca but may depend on the aspect

ratio of the rectangular channels in the cross-junction. For higher values of Ca , e.g., $Ca \geq 10^{-3}$, Eq. 4.30 reduces to the classical squeezing law (Eq. 4.2). Curve fitting of Eq. 4.30 to the experimental data shows excellent agreement, as illustrated in Fig. 4.23. The data series were obtained from cross-junctions with rectangular channels of aspect ratios $W/H = 0.95, 1.43, 2.04, 3.05$, and 4.07 . The resulting dimensionless fitting parameters of each device are listed here. $W/H = 0.95$: $\{\chi = 1.62 \times 10^{-2}, p = 0.68, \lambda_0 = 1.98, \phi = 4.87 \times 10^{-4}\}$, $W/H = 1.43$: $\{\chi = 1.72 \times 10^{-2}, p = 0.49, \lambda_0 = 1.68, \phi = 3.52 \times 10^{-4}\}$, $W/H = 2.04$: $\{\chi = 1.78 \times 10^{-2}, p = 0.74, \lambda_0 = 1.64, \phi = 2.70 \times 10^{-4}\}$; $W/H = 3.05$: $\{\chi = 3.46 \times 10^{-2}, p = 0.41, \lambda_0 = 1.63, \phi = 1.69 \times 10^{-4}\}$, $W/H = 4.07$: $\{\chi = 2.41 \times 10^{-2}, p = 0.97, \lambda_0 = 1.65, \phi = 0.89 \times 10^{-4}\}$.

Some previously reported measurements have shown similar behavior at the low- Ca range as in the current study. For example, the experimental result of Cubaud and Mason [28] shows two different droplet formation regimes, distinguished by threshold value of $l_D \approx 2.5$, where for $l_D > 2.5$, l_D increases by proportionality $\propto Ca^{-1}$. This result can be readily explained by the mechanism that has been described here; for example, Eq. 4.30 shows the proportionality of $l_D \propto Ca^{-1}$ confirming the experimental data in [28].

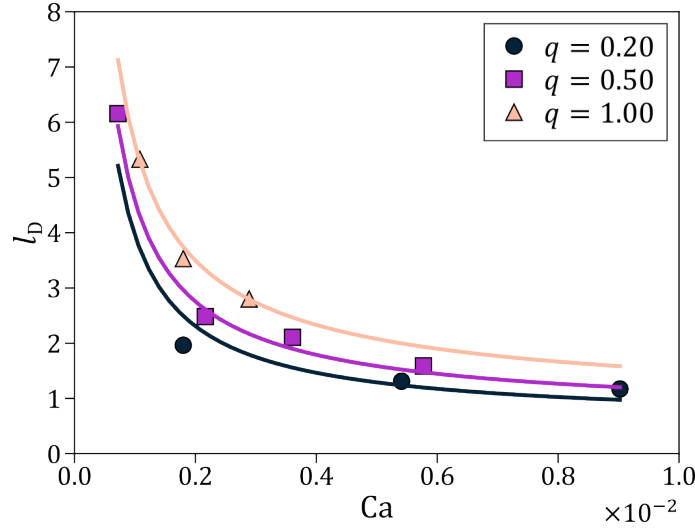


Fig. 4.24: Points are the experimental results from [58]. Solid lines are the curve-fitting result of Eq. 4.30. The liquid pair is CP: deionized water and DP: PDMS silicone oil with a viscosity ratio $\mu_D/\mu_C = 12$, taken from the device with $W/H = 1$. Further experimental details can be found in [58]. Curve fitting results: $\chi = 6.10 \times 10^{-2}$, $p = 0.12$, $\lambda_0 = 0.48$, $\varphi = 30.50 \times 10^{-4}$

Additional experimental verification of Eq. 4.30 was performed using experimental data from [58], as shown in Fig. 4.33. The liquid set consists of deionized water and PDMS silicone oil as CP and DP, respectively, with a viscosity ratio $\frac{\mu_D}{\mu_C} = 12$. The W/H of the cross-junction device is 1 with a cross-sectional area of $600\mu\text{m} \times 600\mu\text{m}$. The experimental data from [58] was chosen because, albeit limited, it provides a set of values of q , $l_D > 2.5$ and Ca required for the curve fitting of Eq. 4.30. Moreover, the data provided in [58] also includes measurements at the low- Ca range. The solid lines in Fig. 4.33 show the curve-fitting result based on Eq. 4.30, and it shows good agreement with the experimental data.

Moreover, additional experiments were conducted using another liquid pair. n-hexadecane with $\mu_C = 3.6 \text{ mPa s}$ and distilled water with ($\mu_D = 0.95 \text{ mPa s}$) were used as the CP and DP, respectively. The surfactant Span 80 (Sigma-Aldrich) was added to the CP at a 1% wt concentration to avoid wetting of DP to the microchannel. The interfacial tension is 4.8 mN m^{-1} . The points in Fig. 4.25 show the experimental results, whereas the solid lines show the curve-fitting results based Eq. 4.30. The results shown here provide additional evidence for the universality of the generalized droplet formation model, Eq. 4.3, and of the proposed scaling of l_D , Eq. 4.30, which explains the increase in l_D at very low Ca corresponding to the leaking regime existence

as the squeezing regime lower boundary.

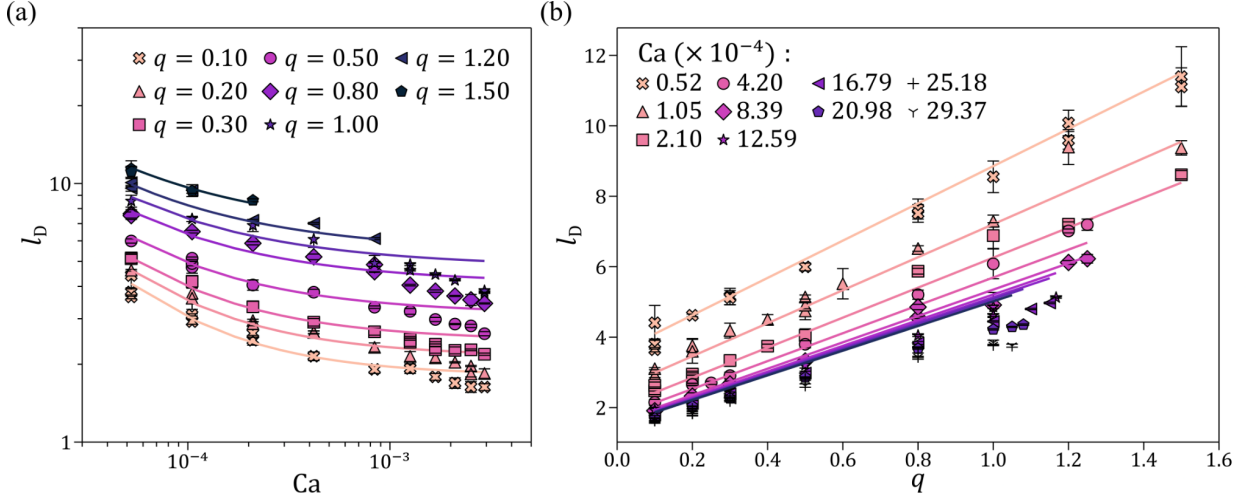


Fig. 4.25: Points are the experimental data using CP: n-hexadecane + 1 wt% surfactant and DP: distilled water with viscosity ratio $\mu_D/\mu_C = 2.6$, measured from the device with $W/H = 4.07$. Solid lines are the curve-fitting result of Eq. 4.30. The same symbol and color show measured l_D of constant (a) q and (b) Ca , respectively. The solid line with the corresponding color represents the theoretical l_D of the same (a) q and (b) Ca . The curve fitting parameters are (all dimensionless) $\chi = 1.50 \times 10^{-2}$, $p = 3.23$, $\lambda_0 = 1.48$, and $\varphi = 1.10 \times 10^{-4}$.

4.2.8 Effect of the channel geometry

4.2.8.1 The gutter geometry in channels of different aspect ratios

The relative cross-sectional area of the gutter is essential for the efficiency of the leaking flow through the corners of the rectangular channel. It is important to understand the gutter geometry, which depends on the channel geometry described by the channel cross-section aspect ratio. Consider the equilibrium shape of a confined droplet in a channel of width W and height H . In the case of equilibrium, the curvature is uniform on the free interface between the two phases. Here, it is assumed that the interface at the front could be estimated by the arcs of the circles inscribed in the channel in two perpendicular planes with radii equal to $W/2$ and $H/2$. Thus, the curvature of the front of a droplet can be estimated as follows:

$$\kappa_f = \frac{2}{W} + \frac{2}{H}. \quad (4.31)$$

In the gutter, one principal radius is the radius of the droplet cross-section, called the gutter radius, R_G , as shown in Fig. 4.26. As the gutter is assumed to be uniform in the direction parallel to the channel, the other principal radius can be assumed as a straight line (radius is $\sim \infty$). Thus, the curvature of the gutter can be estimated as:

$$\kappa_G = \frac{1}{R_G} + \frac{1}{\infty} = \frac{1}{R_G}. \quad (4.32)$$

In the equilibrium state, the pressure difference between outside and inside the droplet is equal everywhere, in which $\kappa_G = \kappa_f$ applies, such that R_G can be estimated as:

$$R_G = \frac{1}{2} \left(\frac{1}{W} + \frac{1}{H} \right)^{-1}. \quad (4.33)$$

Finally, the area of the gutter A_G can be estimated as:

$$A_G = \left(1 - \frac{\pi}{4}\right) R_G^2 = \left(1 - \frac{\pi}{4}\right) \cdot \frac{1}{2} \left(\frac{1}{W} + \frac{1}{H}\right)^{-2}. \quad (4.34)$$

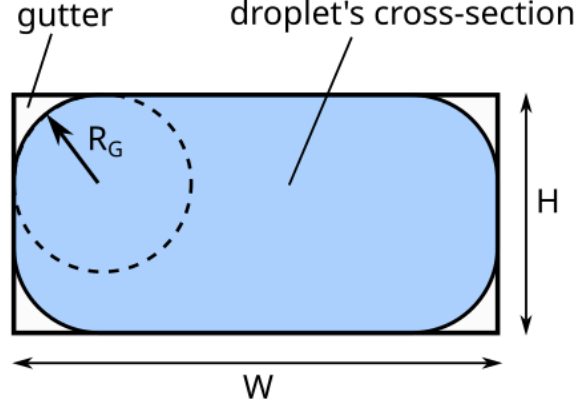


Fig. 4.26: Schematic view of the cross-section through the channel with a droplet confined by channel walls.

4.2.8.2 The leaking strength

As mentioned previously, the fitting parameters of Eq. 4.30 are expected to depend on the junction geometry. Consider the parameter ϕ in Eq. 4.30, which can be thought of as the strength of the leaking flow. The magnitude of ϕ depends on the ratio of capillary to viscous forces. To estimate the effect of the channel geometry, given by the aspect ratio of the channel cross-section (W/H), on the process of leaking, the magnitudes of the pressure loss for gutter flow and for the Laplace pressure drop are compared. For the leaking flow, it is assumed that the gutter has a length equal to W and the gutter flow equal to $Q_C/4$, assuming a uniform distribution of the entire flow Q_C between four gutters. Thus, the pressure drop through the gutter can be written as:

$$\Delta P_G = \frac{1}{4} \frac{\alpha}{A_G^2} \mu_C W Q_C, \quad (4.35)$$

where A_G is given by the Eq. 4.34. Simplifying Eq. 4.35, the characteristic viscous pressure drop across the gutter gives:

$$\Delta P_G \approx \alpha \mu_C U_C \left(\frac{W}{H}\right)^{-1} W^{-1} \left(1 + \frac{W}{H}\right)^4. \quad (4.36)$$

Next, the magnitude of the Laplace pressure drop based on the curvature of the front of the droplet can be estimated as

$$\Delta P_L = 2\gamma \left(\frac{1}{W} + \frac{1}{H}\right) = 2\gamma W^{-1} \left(1 + \frac{W}{H}\right). \quad (4.37)$$

Finally, the ratio of the two pressure drops is written as:

$$\frac{\Delta P_L}{\Delta P_G} \approx \alpha^{-1} \text{Ca}^{-1} (W/H) (1 + W/H)^{-3}. \quad (4.38)$$

The parameter ϕ in Eq. 4.30 is assumed to follow the same dependency on W/H as in the above equation. Thus, the assumption leads to an estimation of ϕ in the form:

$$\phi = c(W/H) (1 + W/H)^{-3}, \quad (4.39)$$

where c is an arbitrary constant, the experimental values of ϕ were obtained from fitting measurements for each device, which is mentioned previously in Section 4.2.7. Fig. 4.27 shows that Eq. 4.39 correctly relates ϕ to W/H . As shown in Figure Fig. 4.27, the leaking strength is lower in the device with a higher aspect ratio, i.e. flatter device.

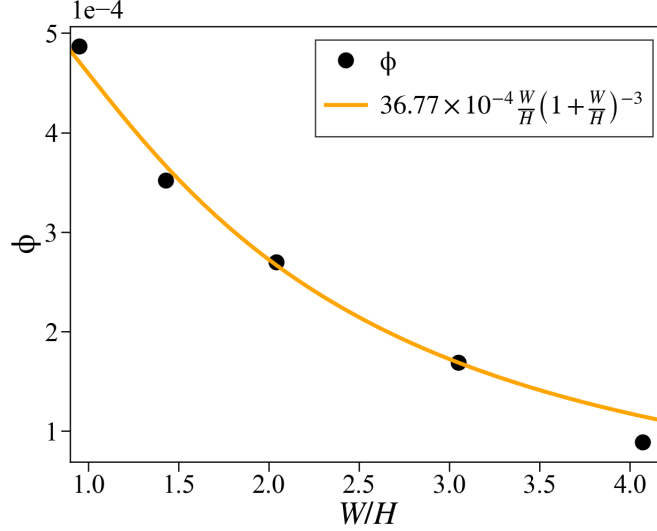


Fig. 4.27: ϕ as a function of device aspect ratio W/H . The flatter the device is (higher W/H), the weaker the effect of leaking flow.

4.3 Droplets as cell incubator

4.3.1 Cell distribution inside droplets

Section 3.4.2 describes how the cell clamping problem was solved using the pipette tip or trimmed steel needle in the input line of DP. The use of a pipette tip or a trimmed steel needle localizes the medium containing cells in close proximity to the cross-junction, reducing the distance traveled. Moreover, the use of a negative pressure driving system greatly reduces the loading time of the medium-containing cells. Thus, both improvements were made to prevent cells from clamping in the inlet line. Without clamping of cells, the encapsulation of cells in a droplet is an independent event, and the probability of such an independent event is governed by the Poisson formula (Eq. 2.19), rewritten here as $P(\lambda, k) = \frac{\lambda^k e^{-\lambda}}{k!}$.

Fig. 4.28 shows the droplets count having k number of cells, $N_D(k)$ obtained from four different experiment results. The average number of cells in a droplet, λ , was calculated by the multiplication of cell concentration in the medium with the volume of a single droplet. In other words, λ represents an ideal scenario in which all cells are evenly distributed throughout the medium, and the bulk medium is instantaneously divided into droplets of uniform size. The experimental measurement is compared with the Poisson distribution, with the parameter λ_{fit} shown as blue lines in Fig. 4.28. All measurements follow the Poisson distribution, proving that there is no formation of cells clamping in the inlet line.

Since an experiment can never achieve ideal conditions, it is anticipated that λ_{fit} will not attain the ideal value λ . What causes the non-ideal conditions? There are a few factors to be considered:

The first factor is associated with cell distribution throughout the medium. Recall that the cell-containing medium was mixed by performing at least three repeated aspirate-dispense cycles with the pipette. The medium is then aspirated into the pipette tip, and then the pipette tip is inserted into the chip-DP inlet, followed by immediate droplet generation. The time from

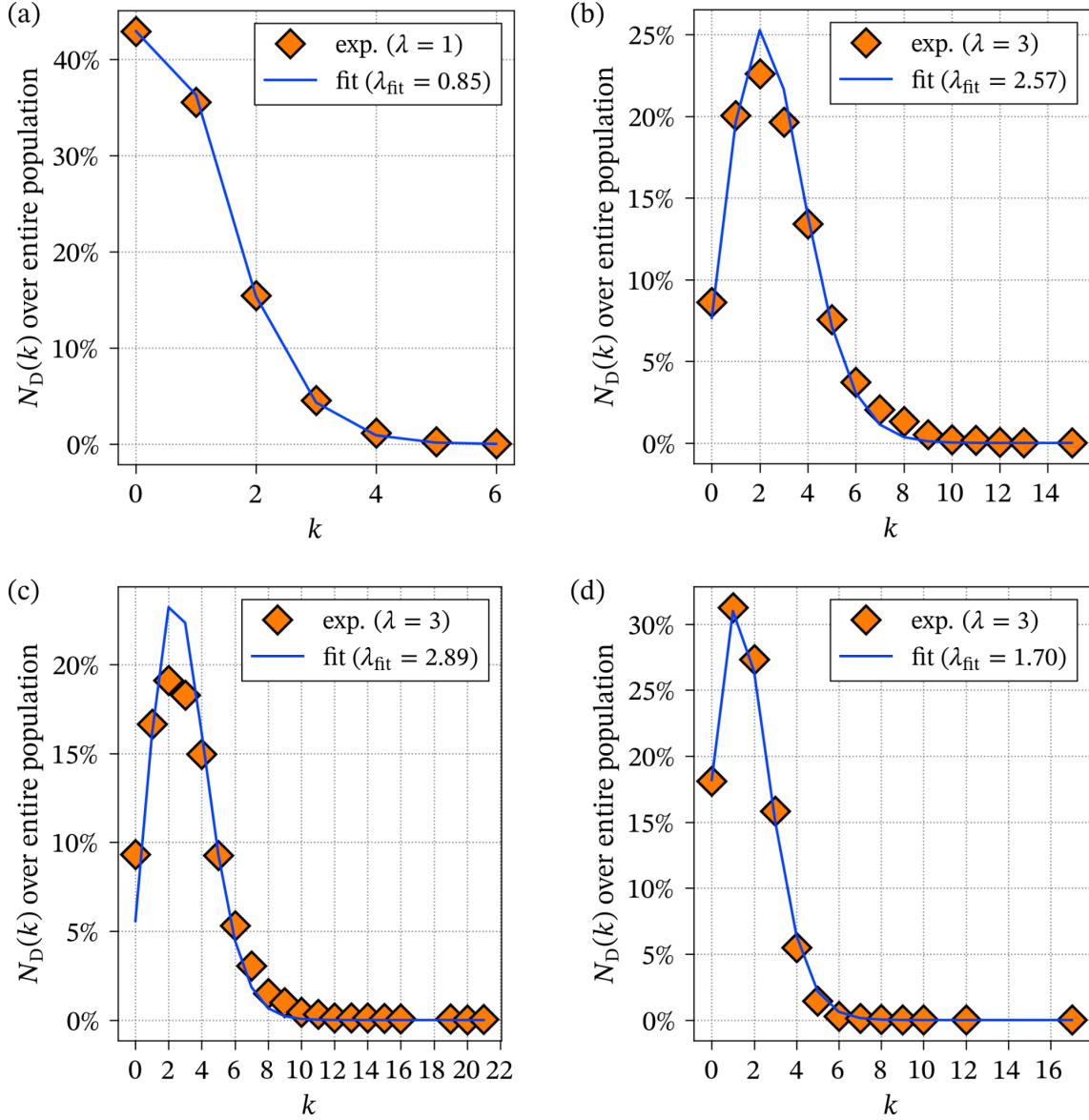


Fig. 4.28: The count of droplets, N_D having k number of cells. The experimental results (exp.) are shown as the orange points. The experimental $\lambda(s)$ were obtained by calculating the average number of cells per droplet volume. The blue lines are the curve fitting line based on Poisson distribution, with the λ_{fit} as the only fitting parameter. The line is not a continuous line because the Poisson distribution is a discrete probability function, where k is a non-negative integer. (a)-(d) shows four distinct experiment results, but all of them show Poisson distribution confirming independent event of cell encapsulation.

pipette tip insertion to the first droplet formation is typically 1–3 min. Although there was an improvement, the cell's concentration can never be perfectly homogenous throughout the medium. The aspirated medium may contain less or higher concentration of cells than the average. The cells are sedimenting to the bottom due to gravity; thus, leaving the medium too long will increase the density of the cells in the bottom part of the pipette tip (or needle steel). Cells aggregate may also form if the medium is left for a prolonged time inside the pipette tip (or needle steel). It is also worth mentioning that cell concentration in the medium is typically higher in the early phase of droplet production compared to the later time, observable by the number of empty droplets during the experiment. The shorter the time between cell loading to droplet production, the less variation of concentration by time observed.

The second factor is the droplet size. Variation of droplet size is very small, and so it has minimum influence on the randomness of the cell encapsulation event; implying that it does not directly affect the actual λ . However, during the beginning of the experiment, typically there is a need for time to adjust the flow rate (i.e., by setting the syringe pump) to obtain the target droplet size. The time required for flow rate adjustment contributes to the total droplet production time, which impacts the cell distribution over the droplets due to variation of cell dispersion within the medium over time. Preventive actions have been taken to reduce this adjustment time:

- Experience from previous tests of the same or other chips was always recorded. The record was used to decide the set of operating flow rates for the next experiment.
- The stability of the temperature affects the overall flow rate. Temperature may cause slight expansion to the system, such as the PDMS and tubing. It is also changing the properties of working fluids, such as viscosity and interfacial tension. In the case of a biological experiment, which requires the incubation of cells at 37°C, the chip –filled with the CP, the syringes, and the CP liquid are all left in CO2 incubators overnight. The environment for the microscope was set to 37°C a few hours before the experiment or the day before.
- Removal of air bubbles in the microfluidic system. Air bubbles that are stuck in the system largely affect droplet generation. Although filling of the chip has been done cautiously to prevent bubbles in the system, from time to time, there can be hidden bubbles trapped in the inlet lines. An important indication that there is a hidden bubble in the system that is interfering the droplet production is the unresponsiveness of the droplet size to the variation of flow rate. A system free of bubbles is sensitive to the change of flow rate, for example, increasing the flow rate ratio, $q = Q_D/Q_C$, increases the droplet size. A system with hidden bubbles shows unresponsive droplet generation against change of flow rate, for example, the change in flow rate ratio does not result in a significant change of produced droplet size.

The third factor affecting the actual λ is related to the sampling of the measurements. All measurements shown in Fig. 4.28 are the subset of the total droplet immobilized in the incubator region. A subset of the total incubator area was chosen to be recorded for the biological analysis (while the rest was neglected) to keep the time between frames to a minimum. Although the measurement of all droplets is not allowed in this case, the subset still shows the Poisson distribution. The measurement of all produced droplets, theoretically, will lead λ_{fit} closer to the expected λ .

4.3.2 Droplet pervaporation in different type of chip construction

Although the use of droplets as cell incubators has gained popularity, the chip is typically made of PDMS material, which is known to allow droplet pervaporation, introduced in section 2.6. In this case, the absorption of the medium into the PDMS material is an unwanted effect because it means there is less medium (nutrition) inside the droplet over time. Here, the droplet size measurement of the droplets that are immobilized in the incubator regions is presented for different kinds of chips that were described previously in Section 3.4.3.

4.3.2.1 PDMS-glass chip – common construction

Fig. 4.29 shows the evolution of immobilized droplets in a common chip construction. Recall that a common PDMS-glass chip consists of two main parts: 1) PDMS, where all the channels and structures are with a typical thickness of 3–6 mm, and 2) glass, which typically is a microscopy glass of 1 mm thickness. The two main parts were bonded using plasma treatment (see Section

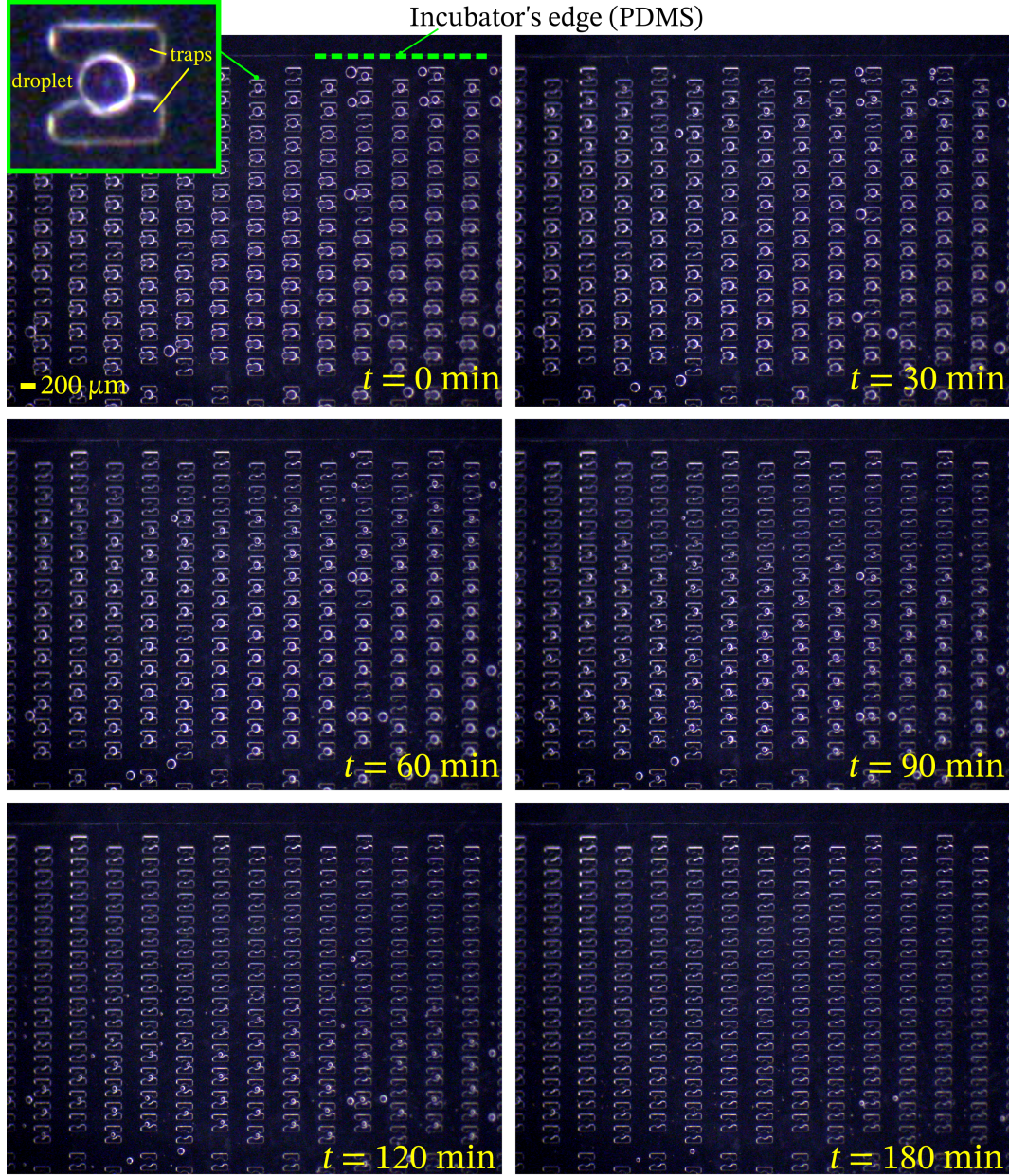


Fig. 4.29: Droplets size evolution by time in common PDMS-glass chip. The closer the droplet is to the edge, the faster it diffuses. The experiment was done in a temperature-controlled room at 21°C.

3.2 for details). The images in Fig. 4.29 show the disappearing droplet over time. Droplets closer to the edge of the chip decrease their size faster than the droplets further away from the edge of the chip. In roughly after three hours, all the droplets are gone. The experiment shown in Fig. 4.29 uses distilled water (mixed with dye) for the DP and fluorinated oil, FC-40, mixed with 2% w/w of surfactant (PFPE-PEG-PFPE) as the CP. The observation was done in a controlled room with a temperature of 21°C. The result shown in Fig. 4.29 triggers interesting questions: what kind of mechanism plays an important role in this disappearing droplet, and how to suppress or eliminate this effect?

There are two mechanisms in play: passive mass transport through permeable material driven by concentration gradient – diffusion and evaporation of the mass in the outer surface of the PDMS. However, which mass transport is more essential is not fully understood, as the process depends on the chip construction. And so, various previous reports have offered different

methods to reduce or eliminate the problem based on their chip construction.

The question inspired a simple experiment using a PDMS slab with some paints. The experiment is elementary, but it can give essential insight. A clean PDMS slab of random size was prepared, and the top side of the slab was painted. Different types of paint (water- and oil-based) were used on different slabs. The PDMS size is irrelevant; however, the current experiment uses the same thickness as the current chip (typically 3–6 mm). Depending on the paint requirement, the slab may be heated in an oven to dry the paint. After the paint dries, the PDMS slab is placed in water without fully immersing it. After some time (less than an hour), the paint shows some wrinkles and, over time, is broken. Although the paint does not completely disallow evaporation, the water molecule that reaches the top of the slab is absorbed or trapped by the paint layer. The simple experiment suggests that the PDMS behaves more like a sponge but with very low permeability. The observation also proposes that a thick PDMS layer, as used in the current experiment, has the diffusion process as a more dominant transport mechanism compared to evaporation. Unfortunately, many previous reports use the term droplet *evaporation* [90, 87, 89], suggesting the evaporation is the dominant mass transport mechanism, while it is not always true. The evaporation might be the dominant flow if the PDMS is thin enough, such that it is possible to be saturated with water, subject to the relative availability of the water on the inner side of the chip. The solution to reduce the evaporation for this thin PDMS is by coating the outer layer of the PDMS with waterproofing polymer [90].

This is confirmed by another failed attempt to reduce the evaporation, that is, by saturating the humidity of the small—closed chamber where the experiment was conducted. The typical experiment uses a small chamber covering the chip with CO₂ oxygen supply to control the amount of CO₂ of 5% inside the chamber over time. Multiple small water tanks were placed inside the small chamber to increase the humidity of the chamber, thus reducing the driving force of evaporation on the PDMS surface. A piece of tissue paper was immersed in the water tank to help the evaporation of water in the small tank. The droplet size still decreases over time at a different rate, but similar to the result shown in Fig. 4.29.

It is also worth noting that the structure in the PDMS part that is made to immobilize the droplets also plays a role in the rate of diffusion of droplets. The incubation chamber with traps as in Fig. 4.29 shows a very fast decrease in droplet size compared to the incubation chamber without any structure. This is logical, as an increase in the surface area of the droplet in contact with the PDMS surface leads to a higher diffusion rate.

Additionally, repeated use of the same PDMS-glass chip – without any drying process of the chip– may result in a decrease in the droplet diffusion effect. This is probably due to the increase in water or medium saturation inside the PDMS from the previous experiment.

4.3.2.2 PDMS-glass chip with liquid pool on top

One quick remedy to reduce the diffusion process is by saturating the thick PDMS layer with water. This can be done by placing a water pool on top of the chip, with typical construction shown previously in Fig. 3.9. A similar approach was reported previously in [91] but with a more sophisticated chip construction. One example of the measurement resulting from the chip with a liquid pool on top is shown in Fig. 4.30. The droplet diameter, D , normalized by its initial value $D_0 = D(t = 0)$, shows a decreasing value but does not completely disappear even after about 37 hours. This means that the liquid pool was able to increase the water content in the PDMS slab, and that decreased the droplet diffusion rate to be much lower compared to the common PDMS-glass device. Although the rate was reduced greatly, the water diffusion was not eliminated. The decrease in droplet volume may affect the likeliness of the cells to survive inside an already limited volume of droplet, with the decreasing volume rate proportional to $\sim D^2$. The decrease in droplet volume is very much unwanted in the biological experiment; thus, another solution is required.

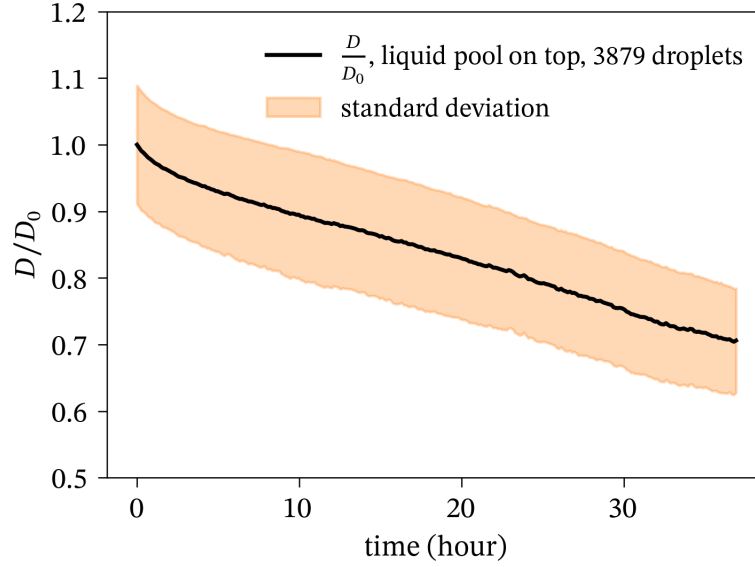


Fig. 4.30: Measurement of droplet diameter D over its initial value $D_0 = D(t = 0)$ in an experiment on a chip with a water pool on top. The droplets are not completely absorbed by the PDMS material as in the previous example (Fig. 4.29), but they remain decreasing over time. The construction of the microfluidic chip was shown previously in Fig. 3.9. Experiments were done in a closed chamber with the temperature and CO_2 concentration at 37°C and 5% respectively.

4.3.2.3 PDMS-glass chip with non-permeable insert and side channels

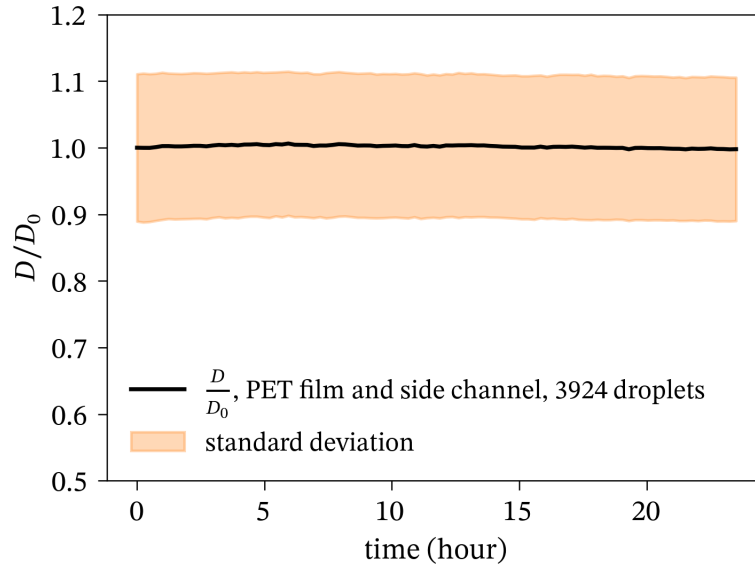


Fig. 4.31: Droplet diameter is almost constant for about 24 hours measured in a PDMS-glass chip with PET film insert and side channel. The device construction was shown previously in Fig. 3.10. The experiment was done in a closed chamber with the temperature and CO_2 concentration at 37°C and 5%, respectively.

Using a liquid pool was proven to reduce the diffusion rate greatly. However, diffusion still occurs, and the reduction of volume may cause problems for the biological substance living inside the droplet. Imagining the PDMS as a sponge, reducing PDMS thickness means reducing the capacitance of PDMS to absorb water. However, reducing the thickness of PDMS causes

additional non-trivial problems:

- Very thin PDMS requires new methods for inlet/outlet connections,
- There must be a minimum thickness of PDMS that allows the removal of PDMS from the mold without breaking the PDMS and ensures the stiffness of the structure. The PDMS layer may expand like a balloon under high pressure if it is too thin. Therefore, the PDMS should still have a thickness of ~ 2 mm, which is quite thick compared to the volume of the droplet. Moreover, it is quite difficult to control the thickness of the PDMS during the fabrication process, resulting in the non-repeatability of the device fabrication.
- The thin PDMS layer may greatly reduce the diffusion transport; however, if there is no prevention of evaporation on the outer surface of PDMS, it may result in a worse evaporation problem.

While reducing the thickness of the PDMS can be ineffective, a simpler solution to reduce the diffusion flow is by placing a thin polymer or glass (non-water-permeable material) plate inside the PDMS layer. After the PDMS was poured, this plate was immersed in the liquid PDMS and placed on top of the incubator area. The example figure of such devices was shown previously in Fig. 3.10. There is still a very thin layer of PDMS beneath the insert plate, which provides passage for gas exchange. The insert plate used in this research is the coverslip (0.50 mm thickness, glass) or transparent PET film (0.2 to 0.4 mm thickness). Both types of plates work as the barrier for diffusion transport, with the latter offering easier fabrication handling. Early experimental results indicate that droplet size is almost constant except for those droplets near the edges of the incubator region. It is logical because there is no barrier of diffusion to the sideways direction. Thus, an additional side channel was added to saturate the PDMS from the sides. The additional side channel (see red color in Fig. 3.10b) does not interfere with the channels of the microfluidic system. The measurement result obtained from the chip with PET film and side channel is shown in Fig. 4.31. In contrast to the previous experiment, the droplet size is almost constant for about 24 hours. It is worth noting that this microfluidic device

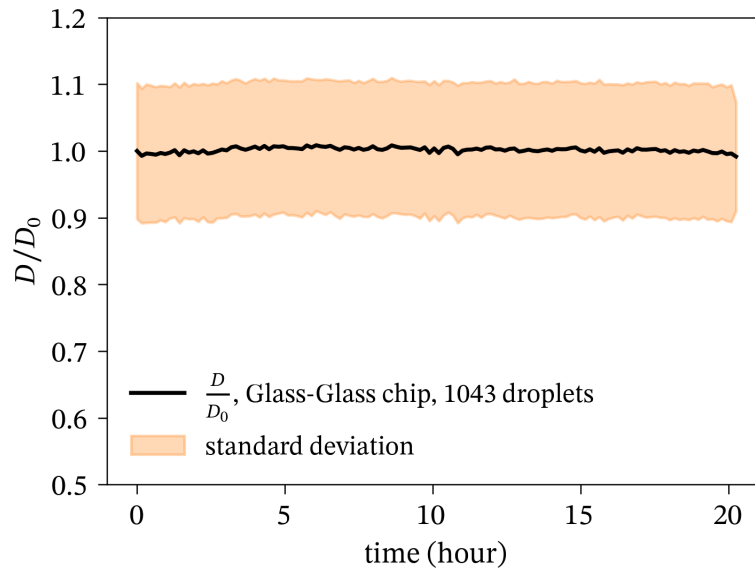


Fig. 4.32: The PDMS material is replaced by a non-water-permeable material: glass, eliminating the pervaporation problem. The chip construction is shown in Fig. 3.11. The experiment was done in a closed chamber with the temperature and CO_2 concentration at 37°C and 5%, respectively.

is without a liquid pool on top and without coating the surface with waterproofing polymer, implying that the evaporation effect is negligible in this scenario.

4.3.2.4 Glass-glass chip

The last type of chip discards the PDMS material as the main part and replaces it with glass, i.e., the glass-glass chip (see Fig. 3.11). The glass material practically eliminates the pervaporation problem; the droplet size is constant over time, as shown in Fig. 4.32. There are two types of glass-glass chip construction, as discussed previously in Fig. 3.11.

4.3.3 The survivability of cells inside a droplet

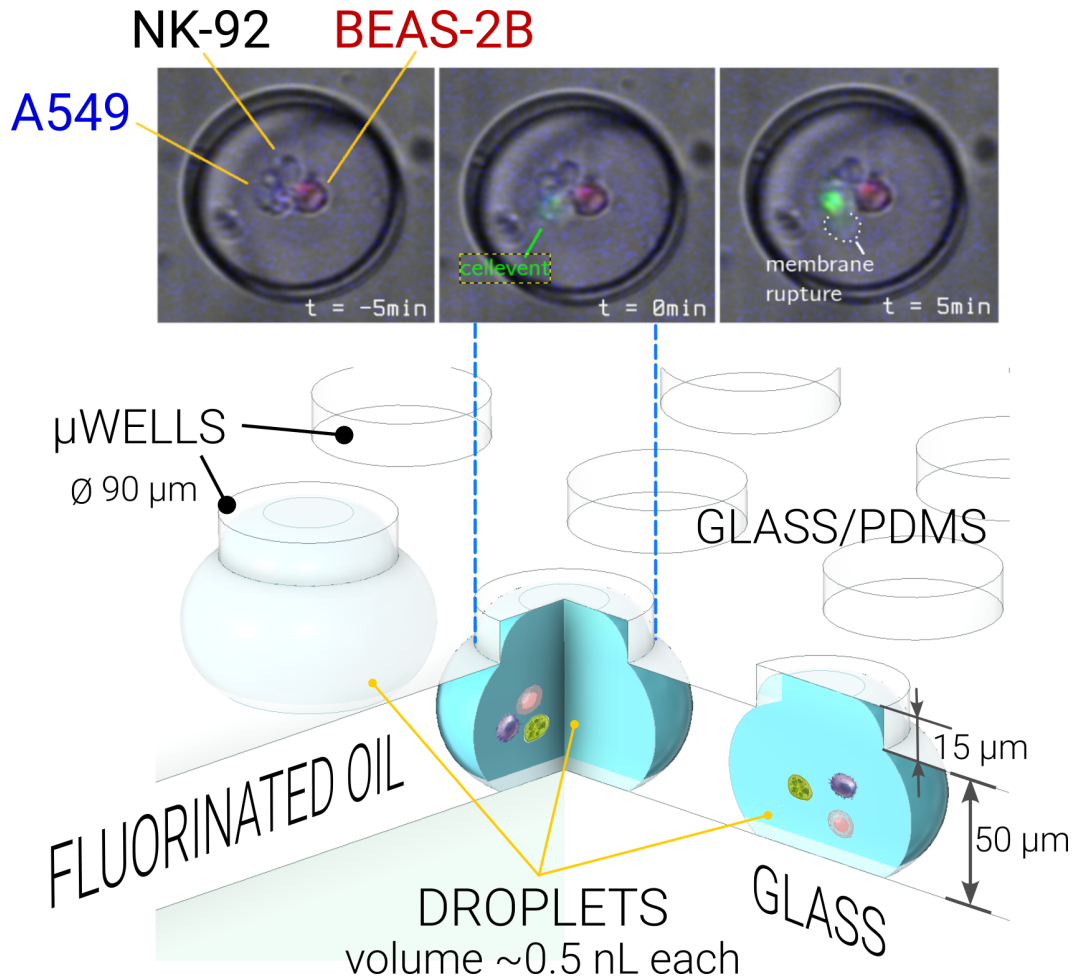


Fig. 4.33: Section view of droplets immobilized in anchors. Each droplet was illustrated to have three different kinds of cells for the biological study, which is out of the scope of the current study. The experimental images on top show the CellEvent, the green-colored signal, which is an indicator of the apoptosis process.

The present section describes shortly about the measurement result that tells about the condition of the cells inside the droplet. The droplets have a volume of around 0.5 nL and are immobilized in the incubator chamber with the glass-glass material. The immobilization of droplets was done by using the μ well anchors [108] with the diameter of 90 μ m that were engraved in the upper glass plate as illustrated in Fig. 4.33. In the upper part of Fig. 4.33, there are experimental images showing an anchored droplet in a μ well consisting of three types of cells: A549 (blue, human lung carcinoma cells), NK-92 (bright-field, natural killer cells) and BEAS-2B (red, normal human lung cells). At $t = 0$ s, the A549 emits a green color i.e., the CellEvent

marker indicating the apoptosis process. Apoptosis is a regulated process of programmed cell death that removes damaged or unnecessary cells in a controlled manner. The interaction of NK cells ordering death to the A549 cells is part of the immunology study, and it is outside the scope of the current study. However, before conducting such an immunological study, a preliminary test is needed, i.e., to check if cells, without the killing order, can sustain their life inside the droplet. Only one cell type was used for each experiment, and the same CellEvent marker, as shown in Fig. 4.33, was used for apoptosis detection. For this preliminary test, two sets of experiments were prepared with different cell lines: the A549 cells and the K562. A549 cells are a human lung cancer cell line derived from a patient with lung adenocarcinoma, often used in research to study lung function, cancer, and respiratory infections. K562 cells are a type of human cancer cell line originally derived from a leukemia patient, commonly used in research to study blood cell behavior and cancer treatments. The results for the A549 cells and K562 cells are shown in Fig. 4.34a and Fig. 4.34b, respectively. The measurements of CellEvent were done through images. The cells that are labeled with CellEvent emit a specific wavelength of around 530 nm when they start the apoptosis. This signal is captured by fluorescence imaging. An example of such an image is the green color overlaid on top of the bright field image shown in Fig. 4.33. In an 8-bit grayscale image, when there is no CellEvent signal, the intensity of the whole image is just 0 (black). When there is CellEvent, the signal is converted into the 8-bit value ranging from 0 to 255 (white). Fig. 4.34 shows the detected CellEvent based on different values of the intensity threshold. The lower the threshold, the more low-value signals are recognized as positive CellEvent signals. Fig. 4.34 shows that even with the lowest threshold value, there are only about 8% of cells emitting positive CellEvent signal. This is a good indicator because the majority of cells ($>92\%$) are still alive after more than 18 hours. The results prove that the prepared microfluidic system can provide healthy microenvironments for the living cells, and such a system can be used for biological study, for example, in an immunology study.

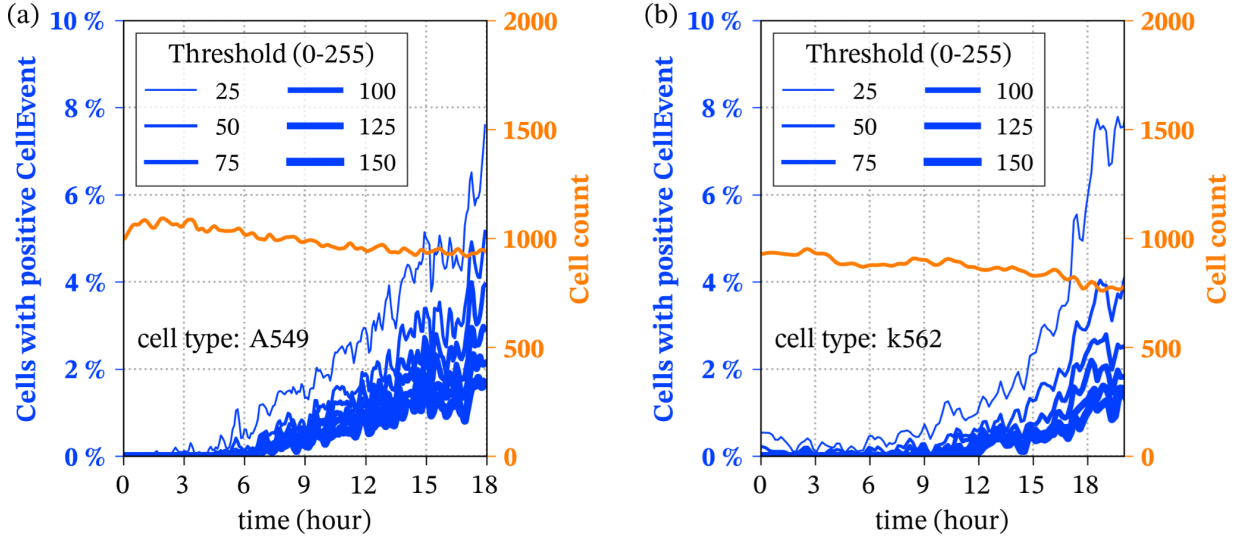


Fig. 4.34: The percentage of (a) A549 and (b) K562 emitting CellEvent signal based on different threshold value. Both results show that only about 8% of cells emitted CellEvent after about 20 hours of the experiment. The results imply that the cells inside the droplets are not dying. Both experiments were done in a closed chamber with the temperature and CO_2 concentration at 37°C and 5%, respectively.

Chapter 5

Conclusion

The formation of droplets at cross-junctions under low capillary numbers (Ca) was investigated. A lower limit of the squeezing regime was identified, below which a strong Ca -dependency on the droplet volume became evident. While similarities in the leaking regime of droplet formation were observed for both cross-junctions and T-junctions, fundamental differences between these geometries were also revealed. These differences arise from constraints on the interface of a forming droplet imposed by the junction geometry.

In T-junctions, the shape of a forming droplet is constrained by the side walls of the outlet channel. As a result, the critical shape at pinch-off depends solely on the total amount of the continuous phase accumulated behind the droplet, independent of Ca . In contrast, in cross-junctions, as demonstrated in this study, the neck shape is not geometrically constrained and elongates spontaneously along the main channel. The neck interface adjusts its shape according to the rate at which the continuous phase accumulates, leading to a critical shape at pinch-off that is dependent on Ca .

Building on these findings, I generalized the classical squeezing law to quantify and rationalize the Ca -dependencies in its parameters. The generalized model shows strong agreement with experimental data.

The primary contribution of this study is experimental and theoretical insight into droplet formation mechanisms in the very low- Ca range, enriching the existing body of knowledge on droplet microfluidics. The findings provide a fresh perspective on the leaking regime in cross-junction devices, though this regime is less favorable due to its sensitivity to Ca . However, the mathematical model developed here can effectively identify the lower boundary of the squeezing regime. Additionally, the stop-flow experiment, a simple yet powerful method, proved useful for validating the neck shape equation. The primarily geometry-based method developed in this work is hoped to inspire future advancements in theoretical droplet microfluidics.

This thesis also includes detailed notes on the soft lithography process using a maskless aligner, which was obtained from the author's extensive fabrication trial-and-error experiences. These technical notes aim to shorten the learning curve for researchers new to soft lithography-based fabrication processes.

In the application part of this study, a microfluidic platform utilizing a cross-junction device was developed and optimized to minimize the loading time of cell-containing media. These improvements involved integrating a pipette tip method and a negative-pressure driving system, significantly reducing loading times. The optimized platform effectively prevented cell aggregation, as confirmed by measurements showing that cell distribution across droplets follows a Poisson distribution.

The study also advances the understanding of water/medium pervaporation in PDMS-glass microfluidic devices. For PDMS devices with typical thicknesses (≥ 3 mm), the dominant mass transport mechanism is the diffusion of water/medium through the permeable PDMS layer. A practical solution to mitigate diffusion involves placing a non-permeable plate (such as polymer

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or glass) over the incubator region, with the optional addition of a side channel surrounding the incubator region.

The developed microfluidic device demonstrated the capacity to sustain most living cells for over 24 hours, providing sufficient time for biological experiments. Overall, the additional thesis results offer practical guidelines for the fabrication and setup of efficient microfluidic systems tailored for biological research.

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