

# PHD NOTES



Rajiv

# CONTENTS

<b>1</b>	<b>Introduction</b>	<b>1</b>
1.1	General Overview . . . . .	1
1.2	Problem Statement . . . . .	3
1.3	Research Objectives . . . . .	4
1.4	Notes Organization . . . . .	5
<b>2</b>	<b>Literature Review</b>	<b>7</b>
2.1	General Overview . . . . .	7
2.2	Salmonella Method of Detection . . . . .	7
2.3	Digital Microfluidics (DMF) . . . . .	8
2.3.1	Architecture . . . . .	9
2.3.2	Droplet Manipulation Techniques . . . . .	10
2.3.3	Core Operations . . . . .	10
	<b>Bibliography</b>	<b>11</b>

# INTRODUCTION

## 1.1 General Overview

The widespread occurrence of Salmonella contamination represents an ongoing public health challenge, causing numerous foodborne illnesses globally. Traditional Salmonella detection methods have significant limitations that prevent quick and precise pathogen identification.

Established techniques like culture-based approaches and PCR are resource-demanding, slow, and sometimes lack the necessary sensitivity for early detection. These methods typically require specialized equipment and trained technicians, restricting their use across different environments, particularly in settings with limited resources or during emergency situations.

There is a clear demand for Salmonella detection systems that are quick, economical, and simple to use, considering the serious health implications of delayed identification. Conventional methods often result in extended response times, potentially facilitating infection spread and hampering effective public health responses.

Additionally, as food supply chains become increasingly interconnected worldwide, there is a growing need for detection technologies that can be readily implemented in various contexts, including laboratories, agricultural settings, and field locations.

To overcome these obstacles, a fundamental shift toward innovative detection approaches is necessary with respect to methods that allows in-situ real-time verification, enhance sensitivity and specificity while also improving accessibility and cost-effectiveness.

Developing a versatile and compact Digital Microfluidics Platform (DMF) with integrated electrochemical (EC) sensors could help identify specific Salmonella bacterial species responsible for outbreaks.

This initiative seeks to enhance public health strategies and enable prompt Salmonella identification, thereby reducing the global impact of foodborne outbreaks.

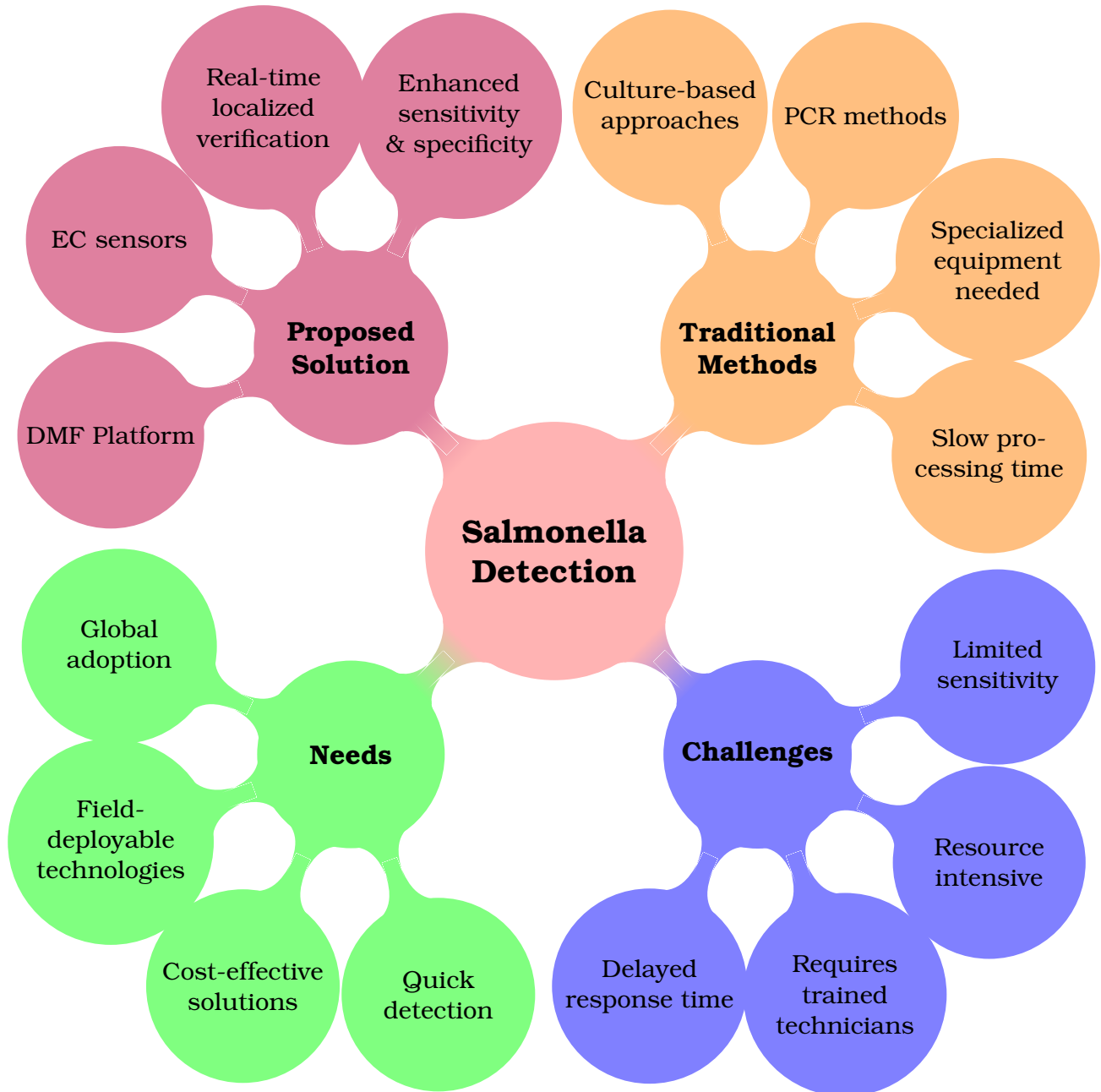


Figure 1.1: Mind map illustrating the challenges of traditional Salmonella detection methods and the proposed Digital Microfluidics Platform solution with integrated electrochemical sensors.

## 1.2 Problem Statement

The persistence of salmonella contamination poses a threat to public health, leading to a significant number of foodborne illnesses worldwide. Identifying which salmonella species contributed to an outbreak are often impeded by the inherent limitations of the conventional methods employed for detecting this pathogen such as time-consuming and labor-intensive procedures [1].

These techniques also require specialized laboratory equipments, trained staff and large volumes of samples, which limits their usefulness in a variety of contexts, particularly those with low resources or in times of emergency [2, 3].

To address these issues, microfluidic techniques have been employed to reduce the volume of samples and reagents needed for bacteria sample preparation, fast reaction and automatic operation [4, 5] prior for detection procedures via electrochemical (EC) or optical sensors.

Microfluidics techniques however involves in designing complex geometries, inclusion of micropumps and microvalves to prepare the samples and produce the necessary results which can be time-consuming [6]. Therefore, it's necessary to find a different approach when creating a platform that can prepare samples and identify *Salmonella* species without relying on extra hardware or intricate geometries.

DMF is a liquid handling technology which manipulates fluids into discrete droplets on a surface of an array of electrodes through non-contact forces such as electrical, magnetic or thermal [4, 5].

Compared to the microfluidics approach, this method would enable sample volumes to be lowered to nanoliters. Additionally, because the generated sample droplets can be precisely manipulated, samples in droplet form can be further divided into smaller droplets for parallel detection.

For bacteria samples identification, optical sensors are often used as compared to EC sensors when combined with DMF-based platform due to the EC sensor lifetime contributed by the rapid dissolution of reference electrode (RE) made up of silver chloride (AgCl) [7].



These systems are, however, expensive and not portable for conducting sample preparation and identification for on-site detection [8]. Hence, this study would investigate and develop a DMF-based platform to be integrated with an array of improved EC sensors to perform *Salmonella* bacteria species detection.

Previous works are concentrated on detecting a specific pathogen from a droplet [8–11] whereby a singular peak detected during the identification process or changes in colour signifies the pathogen existence in the sample. There are, however, less reports on detecting multiple species of a targeted pathogen such as *Salmonella* bacteria.

To address this, a probability method such as Fuzzy Logic can be introduced to help in detecting the probability of *Salmonella* bacteria species existed within the prepared sample. The capability of Fuzzy Logic in determining the pathogen species should be investigated in terms of the sample concentration and voltage resulted from the EC sensor read-out.

### 1.3 Research Objectives

The main objective of this research is to develop a portable and scalable DMF platform for multiple *Salmonella* bacteria species detection. Specific objectives are as follows:

1. To develop a DMF platform for move, merge, split and dispense *Salmonella* bacteria sample in microliter ( $\mu\text{L}$ )
2. To integrate the developed DMF platform with an array of EC sensors
3. To develop and evaluate Fuzzy Logic in determining the presence of *Salmonella* species within a sample.

## 1.4 Notes Organization

The organization of these notes are structured as below;

1. Chapter 1: Introduction

Provides a general overview on salmonella and the detection method, its limitations and how DMF integrated with EC and Fuzzy Logic could provide a solution.

2. Chapter 2: Literature Review

Provides a review on the three topics and critically review the concepts, keypoints, pros and cons, approach and the technology

3. Chapter 3: Methodology

Presents the methods for the project and milestones

4. Chapter 4: Results and Discussion

Presents the findings and discuss the relevancy and whether its corroborated with the literature

5. Chapter 5: Conclusion

Presents whether the objectives has been met





# LITERATURE REVIEW

## 2.1 General Overview

Salmonella bacteria are the pathogens that are capable to cause health related problems such as gastroenteritis and enteric fever, in which an infection normally be caused by contaminated water and food [12–15].

The organization of this literature review notes are structured as below:

1. Section 2.1 provides general overview on salmonella bacteria
2. Section 2.2 provides the available methods (conventional/alternative) used for salmonella detection and the advantages/disadvantages of the said methods.
3. Section 2.3 provides DMF background, timeline of DMF research, and key operations of DMF
4. Section 2.4 provides available DMF fabrication, actuation and validation methods
5. Section 2.5 provides the summary of literature review

## 2.2 Salmonella Method of Detection

Current Salmonella detection methods that are available are listed in Figure 2.1 [16]. Most of these methods required expensive equipments, well-trained personal and no *in-situ* testing [2,3]. Furthermore, the time needed to detect the pathogen ranged between several hours to 4 hours [16].

Due to these limitations, developments of rapid and sensitive detection methods of this particular pathogen has been researched to prevent another outbreak from occurring. The main concern is the modularity and compatibility with other techniques to have the sample to be tested on site and provide preliminary finding before the sample is sent to the laboratory for more detail analysis.

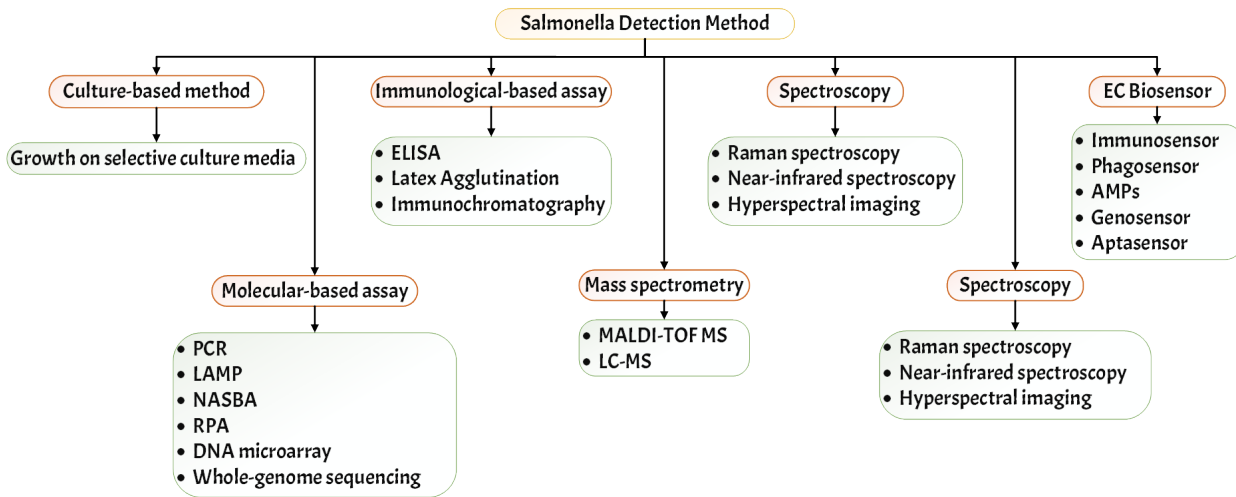


Figure 2.1: Current available methods for Salmonella detection (adapted from [16])

One of the rapid detection methods in the biomedical space is DMF whereby droplets were generated from liquids in microliter ( $\mu\text{L}$ ) and manipulated on an array of electrodes to perform protocols before the droplets are actuated to the sensing block for detection procedures.

This would allow parallel process to be conducted on the same electrodes, which helps in reducing waste and provide economical handling of the samples for on-site testing purposes [17].

## 2.3 Digital Microfluidics (DMF)

Digital microfluidics (DMF) represents a significant departure from traditional microfluidic techniques. Instead of relying on continuous flow through intricate channel geometries and external micropumps, DMF controls liquid movement by manipulating individual droplets on a planar surface using localized electric fields applied on an array of electrodes [18, 19].

Due to smaller form factor, DMF allows smaller liquid volume for laboratory processes to be in microliter ( $\mu\text{L}$ ) and picoliter (pL), thus reducing reagents and samples wastage [20, 21].

Another advantage of DMF have over microfluidics are reduced sample cross-contamination and dispersion. This is due to the droplet served as a self-contained microreactor, there is negligible cross-mixing between different samples or reagents [22, 23].

### 2.3.1 Architecture

DMF are categorically divided into two configurations, which are open and closed as illustrated in Figure 2.2.

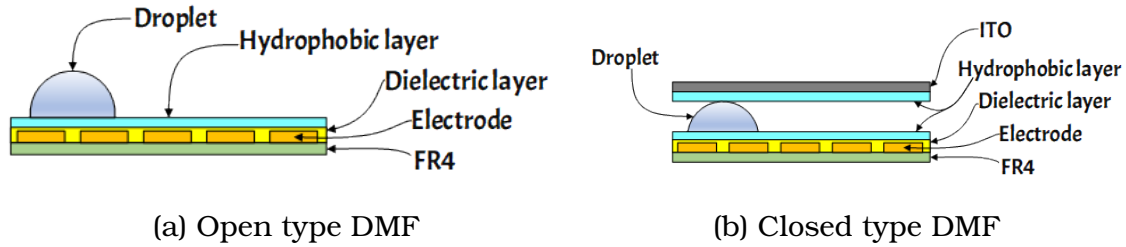


Figure 2.2: Types of DMF configurations using FR4 as the substrate

Open-configuration DMF constitutes of a substrate where an array of electrodes (actuation and grounding connections included), a layer of dielectric layer and a layer of hydrophobic layer are deposited on top of it. Grounding methods for open-type DMF exhibit greater variability and may include a conductive probe inserted into the droplet, exposed ground traces interspersed between actuation electrodes, or subsurface grounding electrodes embedded below the dielectric layer [24]. The absence of an upper confining substrate results in the droplet interface being exposed to ambient conditions or, in certain implementations, immersed in a continuous oil phase to suppress evaporation and enhance droplet stability [25–27].

The dielectric layer electrically isolates the actuating electrode array, thereby suppressing leakage currents and inhibiting electrolysis. A hydrophobic coating is deposited atop the dielectric to lower the surface energy and minimize droplet pinning [17, 18, 28, 29]. Droplet actuation and transport are achieved through modulation of the interfacial forces under an applied electric field.

In the closed configuration, droplets are confined within the gap between two parallel substrates [30, 31]. The lower substrate incorporates a patterned electrode array for actuation, while the upper substrate—typically a continuous ground plane—is often fabricated from a transparent conductive material such as Indium Tin Oxide (ITO) to enable optical observation [28, 32].

Both the actuation electrodes on the bottom substrate and, in many cases, the ground electrode on the top substrate are coated with a dielectric layer to isolate the droplet and preventing electrolysis and dielectric breakdown. A hydrophobic coating is further applied to both surfaces to reduce contact angle hysteresis and enhance droplet mobility [17, 33, 34].

## 2.3.2 Droplet Manipulation Techniques

### 2.3.3 Core Operations

Generally, key droplet operations that can be executed by DMF are shown in Figure 2.3 [35–37].



Figure 2.3: DMF key operations

## BIBLIOGRAPHY

- [1] G. B. L. Silva, F. V. Campos, M. C. C. Guimarães, and J. P. Oliveira, "Recent Developments in Lateral Flow Assays for Salmonella Detection in Food Products: A Review," *Pathogens*, vol. 12, no. 12, p. 1441, Dec. 2023.
- [2] Y. Shen, L. Xu, and Y. Li, "Biosensors for rapid detection of *Salmonella* in food: A review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 20, no. 1, pp. 149–197, Jan. 2021.
- [3] M. Wang, Y. Zhang, F. Tian, X. Liu, S. Du, and G. Ren, "Overview of Rapid Detection Methods for Salmonella in Foods: Progress and Challenges," *Foods*, vol. 10, no. 10, p. 2402, Oct. 2021.
- [4] W. Qi, L. Zheng, S. Wang, F. Huang, Y. Liu, H. Jiang, and J. Lin, "A microfluidic biosensor for rapid and automatic detection of Salmonella using metal-organic framework and Raspberry Pi," *Biosensors and Bioelectronics*, vol. 178, p. 113020, Apr. 2021.
- [5] T. Nguyen, A. Chidambara Vinayaka, D. Duong Bang, and A. Wolff, "A Complete Protocol for Rapid and Low-Cost Fabrication of Polymer Microfluidic Chips Containing Three-Dimensional Microstructures Used in Point-of-Care Devices," *Micromachines*, vol. 10, no. 9, p. 624, Sep. 2019.
- [6] F. Su, K. Chakrabarty, and R. Fair, "Microfluidics-Based Biochips: Technology Issues, Implementation Platforms, and Design-Automation Challenges," *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems*, vol. 25, no. 2, pp. 211–223, Feb. 2006.
- [7] A. Farzbod and H. Moon, "Integration of reconfigurable potentiometric electrochemical sensors into a digital microfluidic platform," *Biosensors and Bioelectronics*, vol. 106, pp. 37–42, May 2018.
- [8] S. Anderson, B. Hadwen, and C. Brown, "Thin-film-transistor digital microfluidics for high value *in vitro* diagnostics at the point of need," *Lab on a Chip*, vol. 21, no. 5, pp. 962–975, 2021.
- [9] A. M. Foudeh, D. Brassard, M. Tabrizian, and T. Veres, "Rapid and multiplex detection of *Legionella*'s RNA using digital microfluidics," *Lab on a Chip*, vol. 15, no. 6, pp. 1609–1618, 2015.

- [10] L. Lu, H. Zhang, F. Lin, L. Zhou, Z. Zhu, and C. Yang, "Sensitive and automated detection of bacteria by CRISPR/Cas12a-assisted amplification with digital microfluidics," *Sensors and Actuators B: Chemical*, vol. 381, p. 133409, Apr. 2023.
- [11] R. S. Sista, A. E. Eckhardt, T. Wang, C. Graham, J. L. Rouse, S. M. Norton, V. Srinivasan, M. G. Pollack, A. A. Tolun, D. Bali, D. S. Millington, and V. K. Pamula, "Digital Microfluidic Platform for Multiplexing Enzyme Assays: Implications for Lysosomal Storage Disease Screening in Newborns," *Clinical Chemistry*, vol. 57, no. 10, pp. 1444–1451, Oct. 2011.
- [12] R. L. Bell, K. G. Jarvis, A. R. Ottesen, M. A. McFarland, and E. W. Brown, "Recent and emerging innovations in *Salmonella* detection: A food and environmental perspective," *Microbial Biotechnology*, vol. 9, no. 3, pp. 279–292, May 2016.
- [13] P. Pashazadeh, A. Mokhtarzadeh, M. Hasanzadeh, M. Hejazi, M. Hashemi, and M. De La Guardia, "Nano-materials for use in sensing of salmonella infections: Recent advances," *Biosensors and Bioelectronics*, vol. 87, pp. 1050–1064, Jan. 2017.
- [14] E. Pacholewicz, H. J. Wisselink, M. G. J. Koene, M. Van Der Most, and J. L. Gonzales, "Environmental Sampling Methods for Detection of Salmonella Infections in Laying Hens: A Systematic Review and Meta-Analysis," *Microorganisms*, vol. 11, no. 8, p. 2100, Aug. 2023.
- [15] L. Wang and M. Pumera, "Recent advances of 3D printing in analytical chemistry: Focus on microfluidic, separation, and extraction devices," *TrAC Trends in Analytical Chemistry*, vol. 135, p. 116151, Feb. 2021.
- [16] M. S. Awang, Y. Bustami, H. H. Hamzah, N. S. Zambry, M. A. Najib, M. F. Khalid, I. Aziah, and A. Abd Manaf, "Advancement in Salmonella Detection Methods: From Conventional to Electrochemical-Based Sensing Detection," *Biosensors*, vol. 11, no. 9, p. 346, Sep. 2021.
- [17] A. Agarwal and S. Sukhatme, "Digital Microfluidics: Techniques, Their Applications and Advantages," *Journal of Bioengineering & Biomedical Science*, vol. 8, no. 2, pp. 1–6, 2012.
- [18] M. Abdelgawad and A. R. Wheeler, "The Digital Revolution: A New Paradigm for Microfluidics," *Advanced Materials*, vol. 21, no. 8, pp. 920–925, Feb. 2009.
- [19] R. B. Fair, A. Khlystov, T. D. Taylor, V. Ivanov, R. D. Evans, V. Srinivasan, V. K. Pamula, M. G. Pollack, P. B. Griffin, and J. Zhou, "Chemical and



- Biological Applications of Digital-Microfluidic Devices,” *IEEE Design & Test of Computers*, vol. 24, no. 1, pp. 10–24, Jan. 2007.
- [20] B. Bhattacharjee and H. Najjaran, “Droplet position control in digital microfluidic systems,” *Biomedical Microdevices*, vol. 12, no. 1, pp. 115–124, Feb. 2010.
- [21] P. Roy, K. Mondal, M. Kundu, and H. Rahaman, “A new sample preparation technique for linear dilution gradient with minimal sample utilization and waste generation in DMFBs,” in *2015 2nd International Conference on Electrical Information and Communication Technologies (EICT)*. Khulna, Bangladesh: IEEE, Dec. 2015, pp. 205–210.
- [22] Z. Luo, B. Huang, J. Xu, L. Wang, Z. Huang, L. Cao, and S. Liu, “Machine vision-based driving and feedback scheme for digital microfluidics system,” *Open Chemistry*, vol. 19, no. 1, pp. 665–677, Jun. 2021.
- [23] X. Wu, D. Tang, Q. He, L. Liu, Z. Jia, and Y. Tan, “Research progress of electrode shapes in EWOD-based digital microfluidics,” *RSC Advances*, vol. 13, no. 25, pp. 16 815–16 827, 2023.
- [24] M. Yafia, B. J. Emran, and H. Najjaran, “Digital Microfluidic Systems: Fundamentals, Configurations, Techniques, and Applications,” in *Microfluidics: Fundamental, Devices and Applications: Fundamentals and Applications*, 1st ed. Wiley, Feb. 2018, pp. 175–209.
- [25] R. H. Vafaie, H. Dehganpour, and A. Moradpour, “Numerical simulation of EWOD on a printed circuit board for cleanroom-less digital fluidic manufacturing applications,” *COMPEL - The international journal for computation and mathematics in electrical and electronic engineering*, vol. 38, no. 1, pp. 119–137, Jan. 2019.
- [26] M. Yafia and H. Najjaran, “High precision control of gap height for enhancing principal digital microfluidics operations,” *Sensors and Actuators B: Chemical*, vol. 186, pp. 343–352, Sep. 2013.
- [27] M. Yafia, A. M. Foudeh, M. Tabrizian, and H. Najjaran, “Low-Cost Graphene-Based Digital Microfluidic System,” *Micromachines*, vol. 11, no. 9, p. 880, Sep. 2020.
- [28] S. R. Barman, I. Khan, S. Chatterjee, S. Saha, D. Choi, S. Lee, and Z.-H. Lin, “Electrowetting-on-dielectric (EWOD): Current perspectives and applications in ensuring food safety,” *Journal of Food and Drug Analysis*, vol. 28, no. 4, pp. 596–622, Dec. 2020.

- [29] M. J. Jebrail and A. R. Wheeler, "Let's get digital: Digitizing chemical biology with microfluidics," *Current Opinion in Chemical Biology*, vol. 14, no. 5, pp. 574–581, Oct. 2010.
- [30] M. Abdelgawad, P. Park, and A. R. Wheeler, "Optimization of device geometry in single-plate digital microfluidics," *Journal of Applied Physics*, vol. 105, no. 9, p. 094506, May 2009.
- [31] M. Al-Lababidi and M. Abdelgawad, "Minimum movable droplet volume in digital microfluidics depends on the grounding scheme in addition to electrode size," *Sensors and Actuators A: Physical*, vol. 355, p. 114299, Jun. 2023.
- [32] K. Sukthang, E. Pengwang, A. Tuantranont, and W. Wechsathol, "Rapid Fabrication of Close-Typed Electrowetting on Dielectric Devices," *IOP Conference Series: Materials Science and Engineering*, vol. 886, no. 1, p. 012046, Jul. 2020.
- [33] M. J. Jebrail, M. S. Bartsch, and K. D. Patel, "Digital microfluidics: A versatile tool for applications in chemistry, biology and medicine," *Lab on a Chip*, vol. 12, no. 14, p. 2452, 2012.
- [34] A. Meimandi, N. Seyedsadrkhani, and A. Jahanshahi, "Development of an Electrowetting Digital Microfluidics Platform using Low-cost Materials," in *2019 27th Iranian Conference on Electrical Engineering (ICEE)*. Yazd, Iran: IEEE, Apr. 2019, pp. 154–157.
- [35] S. Bhattacharjee, A. Banerjee, and B. B. Bhattacharya, "Multiple Dilution Sample Preparation Using Digital Microfluidic Biochips," in *2012 International Symposium on Electronic System Design (ISED)*. Kolkata, India: IEEE, Dec. 2012, pp. 188–192.
- [36] S. Bhattacharjee, A. Banerjee, T.-Y. Ho, K. Chakrabarty, and B. B. Bhattacharya, "Efficient Generation of Dilution Gradients With Digital Microfluidic Biochips," *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems*, vol. 38, no. 5, pp. 874–887, May 2019.
- [37] B. B. Bhattacharya, S. Roy, and S. Bhattacharjee, "Algorithmic Challenges in Digital Microfluidic Biochips: Protocols, Design, and Test," in *Applied Algorithms*, P. Gupta, C. Zaroliagis, D. Hutchison, T. Kanade, J. Kittler, J. M. Kleinberg, F. Mattern, J. C. Mitchell, M. Naor, O. Nierstrasz, C. Pandu Rangan, B. Steffen, M. Sudan, D. Terzopoulos, D. Tygar, M. Y. Vardi, and G. Weikum, Eds., vol. 8321. Cham: Springer International Publishing, 2014, pp. 1–16.