Microfluidic biochips have gained prominence due to their versatile applications to biochemistry and health-care domains such as point-of-care clinical diagnosis of tropical and cardiovascular diseases, cancer, diabetes, toxicity analysis, and for the mitigation of the global HIV crisis, among others. Microfluidic Lab-on-Chips (LoCs) offer a convenient platform for emulating various fluidic operations in an automated fashion. However, because of the inherent uncertainty of fluidic operations, the outcome of biochemical experiments performed on-chip can be erroneous even if the chip is tested a priori and deemed to be defect-free. Error-Tolerant Biochemical Sample Preparation with Microfluidic Lab-on-Chip focuses on the issues encountered in reliable sample preparation with digital microfluidic biochips (DMFBs), particularly in an error-prone environment. It presents state-of-the-art error management techniques and underlying algorithmic challenges along with their comparative discussions.

- Describes a comprehensive framework for designing a robust and error-tolerant biomedical system which will help in migrating from cumbersome medical laboratory tasks to small-sized LoC-based systems
- Presents a comparative study on current error-tolerant strategies for robust sample preparation using DMFBs and reports on efficient algorithms for error-tolerant sample dilution using these devices
- Illustrates how algorithmic engineering, cyber-physical tools, and software techniques are helpful in implementing fault-tolerance
- Covers the challenges associated with design automation for biochemical sample preparation
- Teaches how to implement biochemical protocols using software-controlled microfluidic biochips

Interdisciplinary in its coverage, this reference is written for practitioners and researchers in biochemical, biomedical, electrical, computer, and mechanical engineering, especially those involved in LoC or bio-MEMS design.
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Sudip Poddar and Bhargab B. Bhattacharya
Dedication

To our parents, teachers, and students
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Foreword

During the past two decades, microfluidic lab-on-chips (LoC) have gained utmost prominence due to their versatile applications to biochemistry and medical science such as point-of-care clinical diagnosis, drug discovery, and synthetic biology. The emergence of this technology has revolutionized medicine by enabling the replacement of bulky biochemical instruments with tiny microfluidic biochips that can automate most of the necessary functions using very little sample fluid and reagents. With the help of in-built sensors, these chips can instantly diagnose a disease just from a few drops of blood or body fluid. An LoC can integrate several laboratory functions on a small chip while providing high-throughput screening and automation. A recent article (2021) published by National Heart, Lung, and Blood Institute, NIH, USA, has announced that these “tiny chips are finally starting to emerge from the lab and are poised to make an impact”. Recent research on the future of LoC businesses also indicates that the consumer market for these chips is extremely promising. It was valued approximately at USD 4 Billion in 2015 and is expected to reach around USD 9 Billion by 2025.

Among different classes of LoCs, digital microfluidic biochips (DMFBs) have become very popular because of their simplicity and ease of operations. They are capable of actuating nano-/pico-liter-sized discrete droplets on a 2D-array of electrodes under electrical control. Thus, they support low-cost and fast implementation of a variety of biochemical protocols. In particular, DMFBs have received wide acceptance due to the flexibility of reconfiguring microfluidic modules while executing multiple operations concurrently on the chip so as to speed up assay-completion time. Sample preparation, which consumes up to two-third fraction of analysis time, plays an important role in almost all biochemical protocols. Dilution and mixing of fluids are two fundamental pre-processing steps in sample preparation. On DMFB architecture, various fluidic operations can be performed on droplets including transport, mixing, splitting, and detection. However, these operations may experience various functional faults (particularly split-errors) during sample preparation, thus impacting adversely the correctness of the application. In particular, volumetric split-errors may occur at any mix-split step of the mixing path due to the inherent randomness of split operations. This poses a significant threat to the reliability of biochemical protocols.

The problem of error-recovery in DMFBs has traditionally been addressed by deploying cyber-physical mechanisms, which are aimed to execute some recovery actions based on the feedback received from on-chip sensors to correct possible volumetric imbalance. Various methods, including checkpointing-based rollback, have been reported in the literature. In this book, the authors have reviewed the existing techniques for achieving error-tolerance including the most recent ones, particularly those suited for reliable sample preparation. New approaches, such as “roll-forward” error correction and sensor-free error-oblivious split operations that lead to the production of target-droplet with correct concentration factors, have been discussed.
Dilution preparation “on-demand” is another important area, where requests for fluid samples with different concentration factors need to be met in real-time. Finally, error management with the most recent class of digital microfluidic biochips known as Micro-Electrode Dot-Array (MEDA) is discussed that guarantees the correctness of the resulting concentration factor without performing any additional rollback or roll-forward action.

Overall, this book is a timely collection of several design optimization techniques that provide the foundation for building error-tolerant microfluidics biochips. Numerous related articles that appeared in journals and conference proceedings in recent years have been diligently reviewed in this monograph. It will serve as a textbook for a graduate course on “Electronic Design Automation (EDA) for emerging technologies”, or as a reference book for researchers and industrial practitioners in the area of LoC and EDA. In a nutshell, the book will help bridge the gap between EDA engineers and biologists and inspire them to explore collaboration in this emerging field.

Taiwan, October 2021

Tsung-Yi Ho, Professor

National Tsing Hua University
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This book is based on several research articles published by the authors in the area of algorithmic sample preparation with digital microfluidic biochips. We are thankful to IEEE and ACM for granting us copyright permission to use the material that appeared earlier in various journals and conference proceedings. We sincerely thank all the co-authors who contributed to these papers and other related work: Krishnendu Chakrabarty of Duke University, USA; Subhas C. Nandy, Susmita Sur-Kolay, Ansuman Banerjee, Pushpita Roy, and Tapalina Banerjee of Indian Statistical Institute, Kolkata, India; Patha P. Chakrabarti of Indian Institute of Technology Kharagpur, India; Sukanta Bhattacharjee of Indian Institute of Technology Guwahati, India; Sudip Roy of Indian Institute of Technology Roorkee, India; Debasis Mitra of National Institute of Technology Durgapur, India; Sarmishtha Ghoshal and Hafizur Rahaman of Indian Institute of Engineering Science and Technology, Shibpur, India; Subhashis Majumder and Nilina Bera of Heritage Institute of Technology, Kolkata, India; Robert Wille of Johannes Kepler University, Linz, Austria; Tsung-Yi Ho of National Tsing Hua University, Taiwan; Juinn-Dar Huang of National Chiao Tung University, Taiwan. Furthermore, we would like to thank all colleagues of Advanced Computing & Microelectronics Unit (ACMU), Indian Statistical Institute (ISI), Kolkata, and the Department of Computer Science and Engineering, IIT Kharagpur, for their constant support and various services. Financial grants from ISI Kolkata, Indian National Academy of Engineering, CSIR and SERB, Govt. of India, for supporting Microfluidic-CAD research at ACMU are thankfully acknowledged. This work has also partially been supported by Linz Institute of Technology, Govt. of Austria. We would like to thank all fellow lab-mates and research scholars of the NRT Lab, ACMU, who have shared their valuable time and enriched us with their ideas. We extend our sincere gratitude to Prof. Tsung-Yi Ho of National Tsing Hua University, Taiwan, who has kindly agreed to write a foreword for this book. Finally, we are thankful to Boris I. Kharissov of the CRC Press and Gabrielle Vernachio and Allison Shatkin of Taylor & Francis Group for encouraging us to proceed with this book proposal.

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Biographies

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Bhargab B. Bhattacharya had been on the faculty of the Computer and Communication Sciences Division at Indian Statistical Institute, Kolkata, since 1982. After his retirement in 2018, he joined the Department of Computer Science & Engineering at Indian Institute of Technology Kharagpur as Distinguished Visiting Professor. He received the B.Sc. degree in Physics from the Presidency College, Kolkata, the B.Tech. and M.Tech. degrees in Radiophysics and Electronics, and the PhD degree in Computer Science, all from the University of Calcutta. His research area includes digital logic testing, and electronic design automation for integrated circuits and microfluidic biochips. He has published more than 400 papers, and he holds ten US Patents. Dr. Bhattacharya is a Fellow of the Indian National Academy of Engineering, a Fellow of the National Academy of Sciences (India), and a Fellow of the IEEE.
Section I

Introduction and Background
1 Introduction

Microfluidics, a technology that enables precise manipulation of small amount of fluid on a tiny chip, has evolved as low-cost and reliable platform for implementing several biochemical protocols, e.g., chemical synthesis, in-vitro diagnostics, drug discovery, and environmental and food toxicity monitoring [138]. Such “micro total analysis systems” (μTAS), also known as “lab-on-chips (LoC)” or “biochips”, offer automation, miniaturization, and integration of complex assays, and were fabricated using fluidic components such as microchannels, micropumps, and microvalves, in the nineties. Originally developed by the semiconductor industry, these devices were later used extensively to build a wider class of Micro Electro-Mechanical Systems (MEMS). LoCs can replace expensive and bulky biochemical instruments, and perform clinical diagnosis, massively parallel DNA analysis, protein crystallization, real-time bio-molecular detection, recognition of pathogens, and immunoassays, in much faster and cheaper ways [158]. They are useful for rapid diagnosis of various diseases including malaria, HIV/AIDS, and neglected tropical diseases (NTD) prevalent in developing countries [88, 116, 145, 152]. These devices can also be deployed for providing immediate point-of-care (PoC) health services [139, 143] and for the management of bio-terrorism threats [63, 170]. Microfluidic technology has recently led to the development of synthetic biology and micro-engineered cell culture platforms, known as organ-on-chip (OoC), that mimics in-vivo environments of living organs [8]. OoCs offer more realistic ambience for modeling diseases, testing the efficacy of drugs, and for the study of prognosis. The International Technology Roadmap for Semiconductors predicted as early in 2007 that medical science would likely to serve as a major driving force in the future [1]. According to a report released by Research and Markets, the global market revenue of in-vitro diagnostics grew from USD 55.8 billion in 2014 to USD 62.6 billion in 2017 and is expected to reach USD 113.1 billion by 2026 from USD 98.2 billion in 2021, at a CAGR of 2.9% during the forecast period [108], a major share of which can possibly be attributed to the emergence of microfluidic LoCs.

Advancements in the biochip industry have revolutionized biological research and life sciences by bringing a complete paradigm shift. Modern microfluidic devices can handle micro/nano/pico-liter volume of fluids, and a typical one is fabricated as a single chip with only a few square centimeters in area [171]. In general, there are two broad categories of microfluidic devices: (i) static 2D microarray of wells, which is usually used for DNA or protein analysis, and (ii) dynamic chips that emulate a sequence of reactions in a controlled fashion. In the latter class, fluids are enabled to move either through a network of tiny channels (flow-based chips), or as discrete droplets actuated on a surface (digital microfluidic biochips). These chips are likely to replace most of the manual and repetitive laboratory procedures in the near future because of their convenience, versatility, portability, and cost. Most of the basic functions that are needed to execute biochemical procedures such as fluid
transportation, merging, mixing, splitting, reaction, analytic separation, and sensing can be supported on such a biochip with the aid of various actuation mechanisms driven by electrical, pneumatic, thermal, acoustic, or optical means. LoCs can thus automate the execution of complex biochemical protocols, reduce reactant cost, process multiple reactions in parallel, with no or little human intervention, thereby eliminating the burden of doing routine tasks and reducing probable errors. Additionally, biochips efficiently perform various operations of a bioassay at much lower cost and with higher speed [27, 139, 169, 171]. One of the most important applications of the biochip is to automate sample preparation, where the major task is to dilute a fluid sample to the desired concentration factor (dilution assay), or to produce a mixture of multiple fluids in a certain ratio (mixing assay).

In the design cycle of integrated circuits (IC), electronic design automation tools have been widely used during the last three decades. In a similar fashion, with the growing complexities of biochip implementation to encompass the diversity of protocols, and with the widening scope for applications, the need for deploying various computer-aided design (CAD) tools and formal methodologies has been strongly felt while optimizing various objectives such as chip area, the layout-map for fluid-transportation network, module placement, assay-completion time, reactant-cost, and route planning for droplet navigation and contamination wash [52,70,127,164–166,186]. These CAD tools also help chip builders to simulate assays, verify design blueprints, validate expected outcomes in the pre-fabrication stage, and test for possible functional and operational errors that might jeopardize correct behavior of the devices, post-fabrication. The work presented in this book discusses most of the recent techniques used for the mitigation of operational errors that might occur during sample preparation with digital microfluidic biochips.

1.1 BASICS OF MICROFLUIDIC LAB-ON-CHIPS

Dynamic microfluidic biochips, based on the principle of liquid propulsion, are broadly divided into two categories, (i) continuous flow-based biochips (CFMBs), and (ii) digital microfluidic biochips (DMFBs). In CFMBs, fluids are manipulated through permanently etched micro-channels with the help of external pressure sources or integrated mechanical micro-pumps [7, 53]. CFMBs can be used to perform a variety of biochemical assays including polymerase chain reaction (PCR), DNA purification, and protein crystallization. However, they often suffer from various fluidic and channel errors, and therefore, ensuring fault-tolerance during sample preparation poses a significant challenge. DMFBs, on the other hand, use electrical actuation to manipulate discrete fluid packets as carriers to implement various fluidic operations such as dispensing, navigation, merging, mixing, splitting, washing, and sensing at the micro-scale [23, 24, 126]. These chips actuate droplets using software-driven electronic controllers [22]. The salient features of DMFBs lie on the controllability of each individual droplet without the need for using micro-channels, micro-pumps, or micro-valves. Hence, various fluidic operations can be performed on DMFBs in a reconfigurable manner, thereby enabling concurrent execution of multiple applications on a single chip. Thus, DMFBs provide
general-purpose programmable microfluidic platforms. Furthermore, error-correcting mechanisms for DMFBs have been well studied in the literature. The detailed architectural descriptions of such biochips along with their applications are described in the literature \cite{23,24,27,39,42,125}. A short review of DMFBs and fluidic operations supported by them are presented in the following section.

1.1.1 DIGITAL MICROFLUIDIC LAB-ON-CHIPS

DMFBs consist of a patterned array of electrodes that can be electrically actuated to perform various fluidic operations \cite{23,24}. This technology is referred to as “digital microfluidics” since one can manipulate discrete-volume droplets on the electrodes in a “digitized” manner. The top-view of a digital microfluidic biochip, and the execution of basic fluidic operations (dispensing, transporting, mixing, splitting, and sensing) are shown in Fig. 1.1(a) for demonstration. By applying time-varying voltage (low/high) to the electrodes, DMFBs can execute a number of bioassays in parallel \cite{147}. DMFBs contain several microfluidic units including input and waste reservoirs, dispensers, mixers, splitters, sensors, thermal units (heater/cooler), as illustrated in Fig. 1.1(a). For example, we have shown two input reservoirs (used for dispensing the droplets) and one waste reservoir (used to drain unnecessary intermediate droplets from the chip). Sensors, which are used to measure concentration, pH, droplet-volume, other physical or chemical properties of fluid droplets, are placed in specified locations on the biochip. Droplet-transportation paths and the location of mixer/splitter modules can be emulated anywhere in vacant regions of a DMFB subject to neighborhood rules, and thus can be dynamically reconfigured on-chip if necessary, e.g., when some electrodes become unusable because of structural degradation or electrical faults.

![DMFB schematic (top-view)](image1)

![DMFB schematic (cross-sectional view)](image2)

Figure 1.1: DMFB schematic (a) top-view and (b) cross-sectional view

The cross-sectional view of a DMFB cell at the detection site is shown in Fig. 1.1(b). Droplets containing biochemical samples are sandwiched between two parallel glass plates (top and bottom) and placed on a hydrophobic surface over an electrode, as depicted in the figure. The top-plate is covered with a single continuous
ground electrode, whereas the bottom-plate is imprinted with an array of controllable electrodes. Silicone oil is used as a filler fluid to fill the gap between the two (top and bottom) plates for preventing droplet evaporation and for reducing surface-contamination. Electrodes are generally connected to control-pins for electrical activation. Light-emitting diodes (LEDs) and photo-diodes are integrated with DMFBs (in fixed positions) for monitoring the status of intermediate or final droplets while executing biochemical assays.

Fluid handling operations such as dispensing, mixing, splitting, and transportation are performed on DMFBs by actuating the droplets in an appropriate fashion using the principle of electrowetting-on-dielectric (EWOD) [126]. Droplets are navigated from one position (source) to another (destination) on the 2-D array when an appropriate sequence of time-varying voltage pulses is applied to control-electrodes to produce a mechanical rolling force along the intended direction. An “electrode actuation sequence” defines the “ON/OFF” switching of an electrode at specific time points. The sequence is determined a-priori to execute a given biochemical experiment [61]. Electrode actuation sequences are generally stored in the memory of a microcontroller or a field-programmable gate array (FPGA). A droplet is moved towards an adjacent electrode when an electric field is applied to the junction of two electrodes. This occurs due to the reduction of the interfacial tension between the liquid and the insulator surface (determined according to the Lippman-Young equation) [115]. For example, the control electrode on which a droplet is resting is turned off, and the left electrode is turned on; the droplet will move towards the left (see Fig. 1.1). Note that the droplet must overlap with a neighboring electrode for successful movement towards it. Droplets are usually not allowed to move diagonally in DMFBs. However, all basic fluidic operations can be performed in any location on the biochip by navigating the droplets toward left, right, north, or south, in controlled fashion (Fig. 1.2). Thus, a DMFB acts as a general-purpose programmable microfluidic platform for implementing a large class of biochemical applications. A biochip is connected to a computer via control pins and receives the necessary actuation sequences for executing an assay. A detailed architectural description of DMFBs and their underlying principles have been described in [27,39,125,149]. DMFBs are best suited for a large class of sample preparation, point-of-care clinical diagnosis, proteomics, and immunoassays, among others.

1.1.2 BIOCHIPS BASED ON MICRO-ELECTRODE DOT-ARRAY (MEDA) ARCHITECTURE

A new architecture for digital microfluidic biochips called “Micro-Electrode Dot-Array (MEDA)” has surfaced lately that aims to overcome certain hindrances associated with conventional DMFBs [176,177]. Some of the limitations of DMFBs are: (i) the inability to vary droplet volume in a fine-grained manner because it is fixed and determined by the electrode-area and actuation voltage, (ii) inability to change droplet shape (typically, spherical) if any need arises to facilitate routing through fluidic obstacles, (iii) availability of a few integrated sensors for detecting droplets in real-time, and (iv) stringent requirements concerning fabrication steps. Unlike