

Algorithmic Challenges in Digital Microfluidic Biochips: Protocols, Design, and Test^{*}

Bhargab B. Bhattacharya[†], Sudip Roy[‡], and Sukanta Bhattacharjee[†]

[†]Nanotechnology Research Triangle, Indian Statistical Institute, Kolkata 700108, India, E-mail: {bhargab, sukanta_r}@isical.ac.in

[‡]Dept. of CSE, Indian Institute of Technology Kharagpur, Kharagpur 721302, India, E-mail: sudipr@cse.iitkgp.ernet.in

Abstract. Recent emergence of microfluidic technology has imparted a profound impact on the implementation of miniaturized healthcare chips and systems. In this review article, we will elaborate on several algorithmic challenges that arise while realizing biochemical protocols on a digital microfluidic (DMF) lab-on-a-chip. In particular, we will focus on certain design automation issues of sample preparation, dilution gradient generation, layout planning, and testing of DMF biochips.

Keywords: Digital Microfluidics, Dilution, Mixing, Sample Preparation.

1 Introduction

In an attempt to mitigate the ever-increasing healthcare costs involved in timely diagnosis of cardiovascular diseases, cancer, diabetes, and global HIV crisis, a new field of interdisciplinary research called “Lab-on-a-Chip (LoC)”, is emerging [1–4]. Typically, an LoC implements a bioprotocol on a single chip that is a few square centimeters in size, which provides a low-cost and fast diagnostic solutions to a variety of medical applications. For on-chip implementation of a bioassay, a fluidic algorithm is needed that will map the corresponding biochemical sequence of the assay to an LoC platform. Research in this new discipline of nanobiotechnology needs the integration of many disciplines, such as microelectronics, biochemistry, pathology, electronic design automation, and fabrication technology, among others. Compared to traditional bench-top procedures, biochips offer the advantages of low sample and reagent consumption, less likelihood of error due to minimal human intervention, high throughput and sensitivity [3, 4]. An ideal on-site biochemical analysis system should be inexpensive, sensitive, fully automated, integrated, and reliable. The emerging application areas of such biochips include, among others, clinical diagnostics, especially, the immediate point-of-care diagnosis of diseases, enzymatic analysis

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(e.g., glucose and lactate assays), DNA analysis (e.g., polymerase chain reaction (PCR), nucleic acid sequence analysis), proteomic analysis involving proteins and peptides, immunoassay, and environmental toxicity monitoring. The continued growth in this emerging field will depend on the advances in algorithmic microfluidics and design automation tools customized for LoCs [4–14].

Microfluidic based biochips are of two kinds. In continuous-flow microfluidic (CMF) biochips, fluids can move through the microchannels fabricated on-chip with the help of the pressure-driven devices like micropumps and microvalves [15]. Digital microfluidic (DMF) biochips, on the other hand, use electrical actuation to manipulate (dispensing, navigation, merging, mixing, splitting, washing, sensing) discrete droplets of nanoliter volume of reactant fluids on a two-dimensional electrode array [4]. Recently, DMF biochips have gained wide acceptance in developing LoC applications.

Mixture preparation of several reactant fluids and dilution of a biochemical sample/reagent are two fundamental preprocessing steps in almost all biochemical assays, such as Blackburn Yeast Colony PCR, Splinkerette PCR, Touchdown PCR, Plant RNA Isolation, Yeast DNA Prep, One Step Miniprep, etc. [16–18]. In these bio-protocols, one may require a reactant fluid with a number of dilutions, or a mixture of several reagent fluids with a given volumetric ratio. For example, in “Yeast DNA Prep”, a mixture requires 2% (v/v) Triton X-100 and 1% (w/v) SDS, where we need to dilute the Triton X-100 fluid to a concentration level as 2% and SDS to a concentration level as 1%. For another example bioassay “Blackburn Yeast Colony PCR”, where a master-mixture is required to be prepared by mixing $2\mu L$ 5X Q-solution, $1\mu L$ PCR buffer, $0.2\mu L$ dNTPs, $0.2\mu L$ forward primer, $0.2\mu L$ reverse primer, $0.1\mu L$ Taq and $5.3\mu L$ water [17]. Thus, here the target ratio of these seven fluids is 20 : 10 : 2 : 2 : 2 : 1 : 53. Similarly, two target ratios of different set of fluids used in “Splinkerette PCR method” are 40 : 10 : 1 : 1 : 48 and 1 : 2 : 3 : 1 : 23 [17].

The basic idea of a microfluidic biochip is to integrate multiple bioassay operations such as detection, sample pre-treatment, and sample preparation on one chip [19]. Front-end functions for sample preparation — *mixing* of three or more different fluids and *dilution* of a fluid with the buffer solution (i.e., mixing of two different fluids), can be done on-chip or outside the chip during the preprocessing steps. Since off-chip sample preparation poses a significant hindrance to the overall bioassay completion time, for fast and high-throughput applications, sample preprocessing steps should also be automated on-chip, i.e., integrated and self-contained on the biochip itself. In this review article, we will discuss some design automation challenges and solutions of on-chip sample preparation, which will lead to an efficient architectural layout implementation of the biochip.

The remainder of the article is organized as follows: In Section 2, we describe the background of DMF biochips. A brief review of the existing techniques for automatic sample preparation protocols using such chips is provided in Section 3. Advances in dilution techniques and gradient generation methods are presented in Section 4. Some contemporary issues in mixing protocols and their layout

design have been presented in Section 5. In Section 6 we briefly touch upon several testing issues in DMF biochips. Finally, in Section 7 we provide some concluding remarks.

2 Basics of Digital Microfluidic Biochips

A schematic diagram of the top view of a DMF biochip is shown in Fig. 1(a) and the cross-sectional view of a basic cell at a detection site of a DMF biochip is shown in Fig. 1(b). A unit cell in the array includes a pair of electrodes that acts as two parallel plates. The bottom plate contains a patterned array of individually controlled electrodes, and the top one consists of a single grounded electrode. The droplets are sandwiched between the two plates, and they rest on a hydrophobic surface over the bottom electrodes.

Droplet movement is achieved by controlling the wettability created due to the interfacial tension (surface tension) gradients between a conductive fluid and a solid electrode with an applied electric field between them. The droplet is moved to the activated electrode by applying a control voltage (above a threshold voltage) to an electrode adjacent to the droplet and, at the same time, deactivating the electrode just under the droplet. For liquids with high surface tension, a special phenomenon called electrowetting-on-dielectric (EWOD) effect [20, 21] is observed on DMF biochips [3]. A DMF biochip may contain several modules, like mixers, splitters, detectors, waste reservoirs, dispensers, and sensors. By varying the patterns of control voltage activation, many fluid-handling operations such as merging, splitting, mixing, and dispensing of droplets can be executed. In addition to electrodes, optical detectors such as LEDs and photodiodes are also integrated in microfluidic arrays to monitor colorimetric bioassays. Detailed descriptions of a DMF biochip and the four fundamental fluidic operations (dispensing, transporting, mixing and splitting) can be found in [1, 20, 22, 23]. For the control of DMF biochips, automation for the user, and technology exploitation, there is a pressing need for advances in computer-aided design algorithms and techniques.

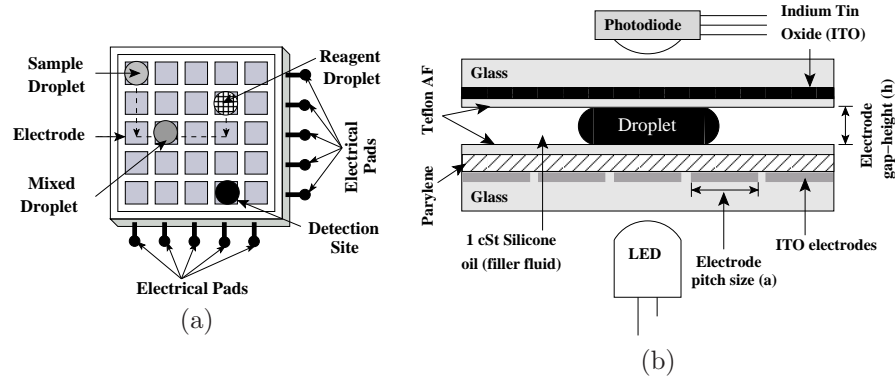


Fig. 1. (a) Top view of a DMF biochip and (b) Cross-sectional view of a cell at a detection site [4].

3 Protocols for Sample Preparation

3.1 Mixing Models

DMF biochips typically work with discrete droplets on a uniform two-dimensional array of equi-sized electrodes, hence their volumes are always integral multiples of one unit volume of a single droplet. A survey on such balanced mix/split modules can be found in [24, 25].

In $(k : \ell)$ *mixing model* (where k and ℓ are non-zero positive integers), k -unit volume of one fluid is mixed with ℓ -unit volume of another fluid to produce $(k + \ell)$ -unit volume of resultant mixture fluid in a single mixing operation. Three different cases may be possible as follows: (i) $k = \ell = 1$, (ii) $k = \ell \neq 1$, and (iii) $k \neq \ell$. The first case, i.e., $(1 : 1)$ mixing model is easy to implement.

In $(k : k)$ *mixing model*, where $k \geq 1$, a DMF biochip needs a module that is capable of mixing two equal (one or more unit) volume fluid droplets or splitting a larger (two or more unit) volume droplet into two equal (one or more unit) volume droplets. Most of the prior work assumed the $(1 : 1)$ *mixing model*, in which one unit-volume droplet of each of two type of fluids are mixed to produce two unit-volume mixture fluid. After each mix operation, the two unit-volume mixture can be split equally into two unit-volume droplets by a split operation. Next, one unit-volume droplet of the mixture is used in the next mixing step, while the other one is discarded as *waste droplets*. One mix operation and a subsequent split operation are together called a *mix-split cycle*.

In the $(k : \ell)$ *mixing model*, where $k \neq \ell$ and $k, \ell \geq 1$, k unit-volume droplet(s) of one fluid is (are) mixed with ℓ unit-volume droplet(s) of another fluid. After each mix operation, either the $(k + \ell)$ -volume mixture can be split equally into two droplets or an one unit-volume droplet can be separated from that mixture by a split operation.

3.2 Dilution of a Fluid or Mixing of Two Fluids

The fluid with which the sample is mixed for dilution is called the diluent or buffer solution, e.g., water or other liquid that is neutral to the sample (i.e., with 0% concentration of the sample in it). Thus, dilution is the special case of mixing of two fluids, where one of them is a buffer (neutral) solution. In order to quantify the amount of raw sample (100% concentration) during sample preparation, we use the term *concentration factor (CF)*. It is defined as the ratio of the initial volume of the sample to the final volume of the diluted sample. In general, dilution of a sample to a CF C_1 can be achieved by mixing it with the same sample of CF C_2 , where $C_2 < C_1$. The CF of the resultant sample will lie between C_1 and C_2 because, if the samples of CF C_1 and C_2 are mixed in a volumetric ratio of $k : \ell$, then the resulting $(k + \ell)$ unit volume fluid has a CF $C_r = \frac{k.C_1 + \ell.C_2}{k + \ell}$.

Dilutions are commonly used in biological studies to create a variety of concentration levels of the reactant fluids on a microfluidic device [26]. Concentrations can extend over a linear or non-linear range and they are generated by two

types of dilution methods such as *linear* or *serial* dilution [26]. In the case of *linear dilution*, the stock solution and its diluent are mixed in different ratios, and this process can create linearly varying concentrations of the stock solution [26]. On the other hand, in *serial dilution*, a common example of which is the logarithmic method, a sample is repeatedly diluted using the same mixing ratio, e.g., 1 : 1. Three serial dilutions mixed at 1 : 1 yield the concentrations $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$. Thus, the serial dilution creates discrete non-linear concentration values of the stock solution.

Recently, many dilution devices and schemes have been demonstrated in the literature for continuous-flow microfluidics (CMF) [27, 28], in order to generate various concentration gradients. However, in the case of DMF biochips, fluids can only be mixed using discrete volumes of droplets. Therefore, for DMF biochips, it is possible to carry out only serial dilution to achieve or approximate desired non-linear concentration levels. Thus, it is a challenge to achieve a desired concentration of the stock solution within a minimum number of mix-split steps with minimum error in target *CF*. However, only a few research articles were reported to date on automatic dilution in DMF biochips [15, 29–31]. An automated DMF-based protocol for extracting proteins from heterogeneous fluids by precipitation has been explained in [32]. This method requires several reagents with different concentration levels, such as 20% TCA (precipitant), 70/30 v/v chloroform/acetonitrile (rinse solution) and 100 mM borate buffer containing 1% SDS (resolubilizing buffer). Another example of a real assay is enzymatic glucose assay (Trinder’s reaction); it uses a dilution factor of 200 or more [29].

Two basic kinds of dilution are: *exponential dilution* and *interpolated dilution*. If a sample is recursively diluted by the buffer solution taking equal volume of both of them for mixing, then the concentration of the sample changes exponentially by a factor of 2. That is, after d cycles of mixing and balanced splitting, the *CF* of the sample changes from C to $\frac{C}{2^d}$, i.e., the *DF* becomes 2^d . This type of dilution is called *exponential dilution*. If a sample of *CF* C_1 is diluted with its another *CF* C_2 taking equal volume of both of them for mixing, then the final *CF* of the sample becomes $\frac{C_1+C_2}{2}$. This is called *interpolated dilution*. Hence, for example, if two droplets of *CF*s $\frac{0}{1024}$ and $\frac{1024}{1024}$ are mixed, then the resultant *CF* after exponential dilution becomes $\frac{1}{2}$ (i.e., *DF* = 2). Again, if two droplets of *CF*s $\frac{1022}{1024}$ and $\frac{1024}{1024}$ are mixed, then the resultant *CF* after interpolated dilution becomes $\frac{1022+1024}{2 \cdot 1024}$, i.e., $\frac{1023}{1024}$.

An (1 : 1)-*dilution tree* is the binary tree representation of the sequence of (1 : 1) mix-split steps required to achieve the desired *CF* of a fluid with the help of buffer fluid. Let T_{ms} be the total number of (1 : 1) mix-split steps in the dilution tree and W be the total number of waste droplets generated in producing two target droplets in the process. Given the desired *CF* C_t , the algorithm *twoWayMix* [15] converts C_t into a d -bit binary fraction and then scans the bits from right-to-left to construct the dilution tree of depth at most d . Each mix-split step combines two unit-volume fluids and outputs two units of their mixture. This algorithm has the advantage that it does not require any storage unit to store intermediate droplets; only the current droplet and the

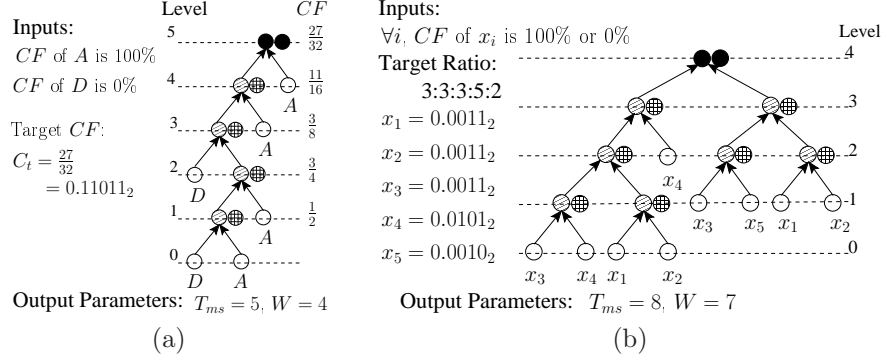


Fig. 2. (a) An $(1 : 1)$ -dilution tree of depth $d = 5$ denoting the sequence of five $(1 : 1)$ mix-split steps for target CF $C_t = 84.375\%$ ($\approx \frac{27}{32} \equiv 0.11011_2$) obtained by *twoWayMix* [15] using fluid A (100% concentration) and buffer (0% concentration). (b) An $(1 : 1)$ -mixing tree obtained by *MinMix* [15] and bit-representations of the example ratio $3 : 3 : 3 : 5 : 2$.

input fluids are required in the next step. However, this method works only when the initial volumes are given with $CF = 0$ (buffer) and $CF = 1$ (100% concentration) sample. Dilution of a sample fluid from the supply of any two arbitrary CF s of that was left as an open problem in [15].

In the literature, a few articles have been reported on automatic dilution of a fluid using DMF biochips [29, 30]. Ren et al. [29] described an experimental study of on-chip dilution of a sample producing 38 integer dilution factors (in the range 2 to 64, given the constraint that only 64-fold exponential dilution and 16-fold interpolating dilution were available) in 10 mix-split steps by interpolating serial dilution method. However, no algorithmic scheme was presented for determining the mix-split steps to achieve the target CF from the input fluids. Griffith et al. [30] first proposed a dilution algorithm of $\mathcal{O}(d^3)$ time complexity, to determine a d -length sequence of mix-split steps for producing two target droplets, given an error tolerance of $\frac{1}{2^{d+1}}$ in the desired CF . It used a binary-search strategy to determine the required dilution steps as a directed graph. However, the design of a suitable architectural layout of electrodes for efficient implementation of the above-mentioned algorithm was left as an open problem.

3.3 Mixing of Three or More Fluids

In many bioprotocols, *mixture preparation* of three or more fluids with a desired ratio, is required. An $(1 : 1)$ -*mixing tree* is a binary tree representation of the sequence of $(1 : 1)$ mix-split steps for the mixture preparation of several fluids. In an $(1 : 1)$ -mixing tree, each leaf node corresponds to a unit-volume droplet and an internal (or non-leaf) node denotes the resultant mixture obtained by applying an $(1 : 1)$ mix-split step on two unit-volume fluid droplets corresponding to its two children. An internal node denotes one unit-volume droplet of the resultant mixture, which is used in the next mix operation denoted by the parent node of the considered internal node. The mixture denoted by an internal node can

only be produced when the mixtures denoted by its child nodes are available or already produced. A post-order traversal of the mixing tree provides the sequence of (1 : 1) mix-split steps required to produce the target mixture (of N fluids) denoted by the root of the tree. The total number of non-leaf nodes in a mixing tree, denoted by T_{ms} , is the total number of mix-split cycles required to produce the target mixture from the supplies and the depth of a mixing tree is denoted by d .

The well-known algorithm *MinMix* [15] determines a mixing protocol tree for a given ratio of several fluids. In this method, the required mixing tree, denoting a sequence of (1 : 1) mix-split steps, is determined from the binary bit-representations of the target ratio (corresponding to all constituent fluids). This task graph is to be executed in order to produce the desired mixture droplets. An example (1 : 1)-mixing tree is shown in Fig. 2(b) for a target ratio 3 : 3 : 3 : 5 : 2 of five fluids, and the corresponding binary representations used to construct the (1 : 1)-mixing tree are shown in Fig. 2(c). However, no architectural layout of electrodes was suggested for executing the mixing/dilution scheme on-chip, and the related design problems were left open.

4 Dilution/Mixing with Reduced Wastage

For an example of generating droplets of target CF $C_t = \frac{313}{1024} \simeq 0.30566_{10} \equiv 0.0100111001_2$ using *twoWayMix* [15], it is observed that five sample and six buffer droplets are required. In 10 mix-split steps, total nine waste droplets are generated. An algorithm called Dilution/Mixing with Reduced Wastage (*DMRW*) has been proposed by Roy et al. [33], which aims to reduce reactant wastage compared to *twoWayMix* [15]. The *DMRW* method uses a binary-search strategy to determine the dilution graph based on $(k : k)$ mixing model (where $k \geq 1$). For the example of generating droplets of target CF s $\frac{313}{1024}$, $\frac{127}{1024}$ and $\frac{513}{1024}$, the dilution graphs obtained by *DMRW* are shown in Figs. 3(a), 3(b) and 3(c), respectively.

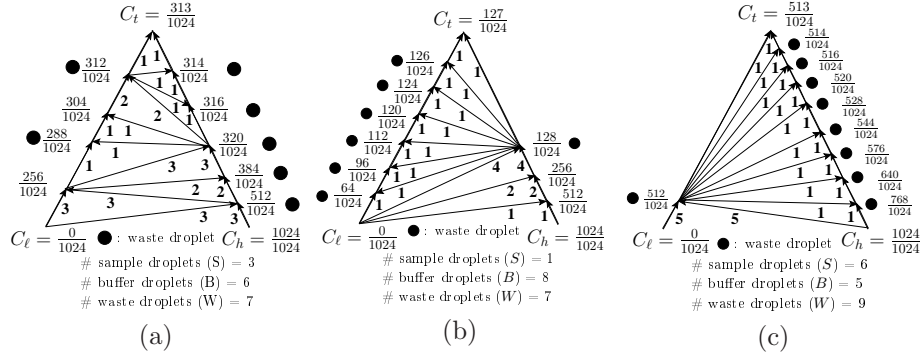


Fig. 3. Dilution graphs denoting the sequence of 10 $(k : k)$ mix-split steps to produce target CF s (a) $C_t = \frac{313}{1024}$, (b) $C_t = \frac{127}{1024}$ and (c) $C_t = \frac{513}{1024}$, obtained by *DMRW* [34].

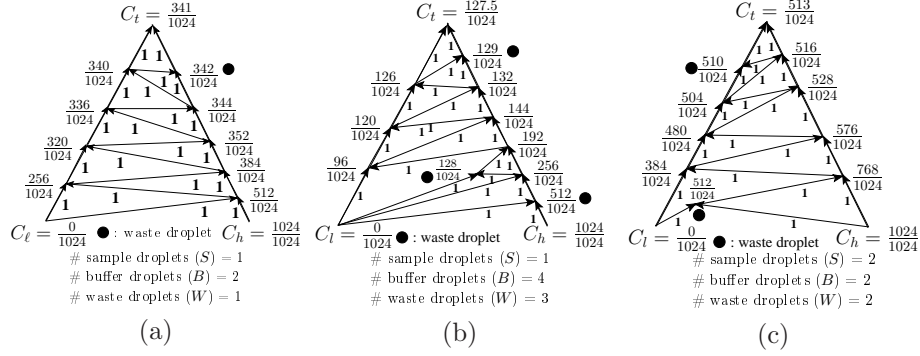


Fig. 4. Dilution graphs denoting the sequence of $(k : k)$ mix-split steps to produce target CF s (a) $C_t = \frac{341}{1024}$, (b) $C_t = \frac{127}{1024}$ and (c) $C_t = \frac{513}{1024}$, obtained by *IDMA* [34].

Subsequently, an Improved Dilution/Mixing Algorithm (*IDMA*) was reported by Roy et al. [34] for further reduction of wastage in the dilution process. It is observed that the outdegree (skew) of a node with a certain intermediate CF value in the digraph obtained by *DMRW* has a strong impact on waste droplet generation as well as on the number of $(1 : 1)$ mix-split steps (e.g., see Figs. 3(b) and 3(c)). In the mix-split digraph obtained by *DMRW*, if the progression sequence towards the target CF alternates between the left(right) and right(left) arms, then the outdegree of each node can never exceed two (e.g., see Fig. 4(a)), and therefore, the waste is optimized and no additional demand of intermediate CF s is created. *IDMA* perturbs the sequence of mix-split steps after a certain step by generating a new intermediate CF without following the binary-search strategy. It can reduce the requirement of input droplets to reduce W furthermore with reduced T_{ms} compared to *DMRW*. The procedure *IDMA* terminates in $O(d)$ steps for producing a target CF with an accuracy of $\frac{1}{2^{d+1}}$. For example, *IDMA* modifies the digraphs obtained by *DMRW* for the two target CF s $C_t = \frac{127}{1024}$ and $C_t = \frac{513}{1024}$ as shown in Fig. 4(b) and 4(c), respectively, and reduces both the values of T_{ms} and W .

Detailed simulation results for $d = 10$, and an architectural layout with two rotary mixers of 16 electrodes, each (Fig. 5), were presented in [33, 34].

4.1 Generation of Dilution Gradients

In sample preparation, producing chemical and biomolecular concentration gradients is of particular interest. Dilution gradients play essential roles in in-vitro analysis of many biochemical phenomena including growth of pathogens and selection of drug concentration. For example, in drug design, it is important to determine the minimum amount of an antibiotic that inhibits the visible growth of bacteria isolate (defined as Minimum Inhibitory Concentration (MIC)). The drug with the least concentration factor (i.e., with highest dilution) that is capable of arresting the growth of bacteria, is considered as MIC. During the past decade, a variety of automated bacterial identification and antimicrobial susceptibility test systems have been developed, which provide results in only few

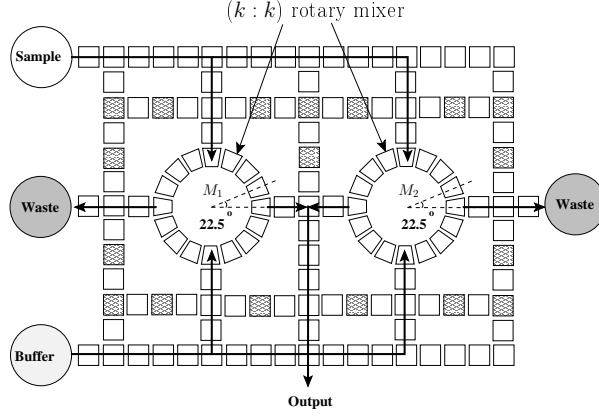


Fig. 5. Layout of electrodes with two DMF rotary mixers for $d = 10$ [33].

hours rather than days, compared to traditional overnight procedures [35]. Typical automated susceptibility methods use an exponential dilution gradient (e.g., 1%, 2%, 4%, 8%, 16%) in which CF s of the given sample are in geometric progression [36]. Linear dilution gradient (e.g., 15%, 20%, 25%, 30%, 35%), in which the CF s of the sample appear in arithmetic progression, offers more sensitive tests. Linear gradients are usually prepared by using continuous-flow microfluidic ladder networks [37], or by other networks of microchannels [38, 39]. Since the fluidic microchannels are hardwired, continuous-flow based diluters are designed to cater to only a pre-defined gradient, and thus they suffer from inflexibility and non-programmability. Also, these methods require a significant amount of costly stock solutions. In contrast, on a DMF biochip platform, a set of random dilution factors can be easily prepared.

4.2 Zero-Waste Linear Dilution Gradients

An algorithm for generating linear dilution gradients on a digital microfluidic platform was reported in [40], which can generate linear gradients without generating any waste droplets. In order to generate linear dilution gradients, this technique extends the target set by adding subsequent linear gradients (depends on the size of the target gradient set to be generated) so that the size of extended target gradient set becomes $2^k + 1$, for a minimum value of k . Next, a full binary search tree representing each gradient is constructed, denoted as Linear Dilution Tree (LDT) following which the required dilution gradient is produced in the postorder sequence of the LDT. The droplets with the concentration values corresponding to each internal node of LDT are used in subsequent mixing operations after their production and are regenerated later for replenishment. In this technique, it is assumed that the two boundary concentrations are available as input droplets. If boundary droplets are not available, they can be efficiently generated using a dilution engine [41]. If the size of target gradient set is $2^k + 1$, then the proposed method can generate target gradients with no waste,

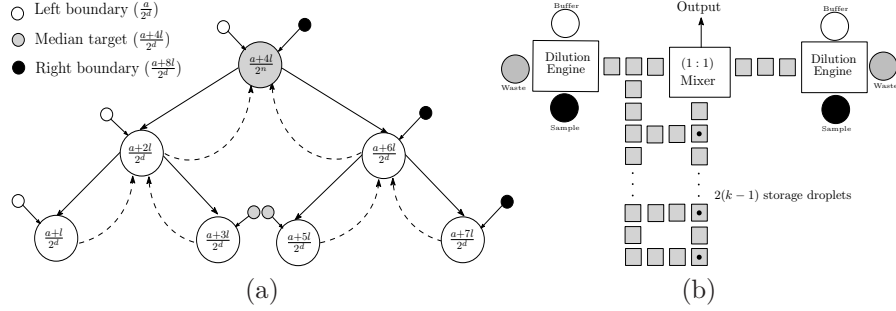


Fig. 6. (a) A linear dilution tree generating 9 linear dilutions $\{\frac{a}{2^d}, \frac{a+l}{2^d}, \dots, \frac{a+8l}{2^d}\}$, where $\frac{a}{2^d}$ and $\frac{a+8l}{2^d}$ are left and right boundary respectively. (b) Architecture layout.

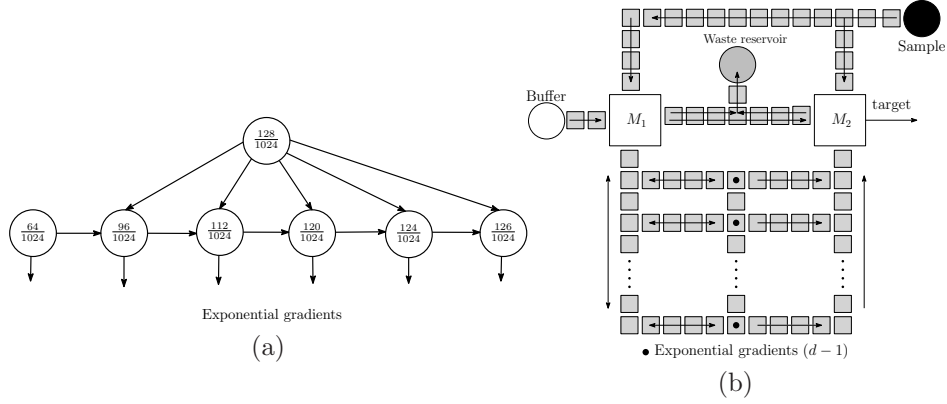


Fig. 7. (a) Sequencing graph for an exponential dilution. (b) Architecture layout.

otherwise a few waste droplets are generated during the process. The detailed performance analysis is given in [40]. An example generating 9 linear gradients is shown in Fig. 6(a), assuming the availability of two boundary droplets $\frac{a}{2^d}$ and $\frac{a+8l}{2^d}$, where a, l, d are the initial term, common difference and accuracy of the linear gradients respectively. An architectural layout is shown in Fig. 6(b), which uses $2(k-1)$ intermediate storage for generating any linear gradient target set of size at most $2^k + 1$. Moreover, two dilution engines [41] are used for supplying the two boundary droplets.

4.3 Exponential Dilution Gradients

The exponential dilution gradient also plays an important role in several biochemical protocols. Using the method REMIA [42], a wider class of exponential dilution gradient generation scheme is proposed recently [43]. An on-chip implementation of an exponential gradient generator on a DMF platform is shown in Fig. 7(b) [43].

5 Mixing Algorithms and Biochip Layout Design

A routing-aware mixing algorithm, known as Ratio-ed Mixing Algorithm (*RMA*) was described by Roy et al. [44]. This method identifies some disjoint *dilution subtrees* in a mixing tree based on top-down decomposition of the underlying algebraic expression of the given target ratio. The (1 : 1)-mixing model is used in this protocol to achieve the target mixture of several fluids. This technique yields a routing-aware layout design that reduces droplet transport time, which, in turn, expedites the execution of the mixing assay compared to the *MinMix* [15]. For the example target ratio 2 : 3 : 5 : 7 : 11 : 13 : 87, the mixing tree generated by *RMA* is shown in Fig. 8(a). The corresponding DMF biochip layout is shown in Fig. 8(b). The required mixing steps can be performed without any crossover among the routing paths of different fluid droplets (as shown in Fig. 8(b)). It is observed that when the mixing tree of *MinMix* is executed, the total number of crossovers among the routing paths becomes nine, whereas, for the mixing tree obtained by *RMA*, this number reduces to four.

Later, a routing-aware Resource (fluid-reservoirs and on-chip mixers) Allocation scheme for Mixture Preparation, referred to as *RAMP* was reported [45]. This algorithm outputs suitable placement of boundary reservoirs and on-chip mixers on a DMF biochip. It was observed that *RMA* along with *RAMP* can reduce the total crossovers among droplet routing paths for 74.6% cases of the total target ratios over *MinMix* along with *RAMP*. Thus, *RMA* along with *RAMP* can reduce the droplet transportation time during on-chip mixture preparation. Subsequently, a few other dilution and mixing algorithms were reported with various optimization goals [13, 41, 42, 46–48].

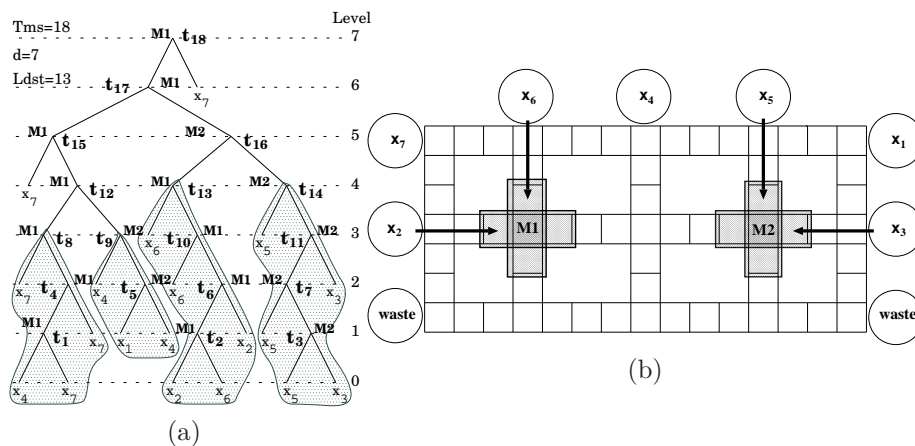


Fig. 8. For the example ratio $2 : 3 : 5 : 7 : 11 : 13 : 87$, (a) $(1 : 1)$ -mixing tree obtained by *RMA* [44], and (b) droplet routes for the mixing steps of the subtree rooted at \mathbf{t}_{16} of the mixing tree.

6 Testing of DMF Biochips

Since microfluidic biochips are often used as life-critical devices, testing of them is very important in order to ensure reliability [49]. Some defects may lead to catastrophic failure of such chips, whereas, a few may cause parametric degradation leading to malfunctioning of certain modules.

Manufacturing test strategies can broadly be classified into two types: structural testing and functional testing [49, 50]. Structural testing targets detection of physical defects, such as, a short circuit between two adjacent electrodes, an open circuit between an electrode and the voltage source. Functional testing aims at identifying the presence of malfunctioning microfluidic functional modules, such as, dispensers, mixers, splitters. Structural testing may be performed for checking droplet movement between every adjacent pairs of electrodes. Such problems of optimal navigation of test droplets can be formulated in terms of Euler tour or Euler trail problems in an undirected graph representing the electrode adjacency structure [51–54]. A DMF biochip that has successfully passed the structural testing does not necessarily ensure correct operation of all of its functional modules such as mixers or splitters [55]. For example, a splitter block of such a DMF biochip may produce two unequal-sized droplets after splitting. Electrodes that support droplet transportation correctly may fail during droplet dispensing from the reservoirs. Several test procedures to detect such defects and malfunctions appear in [54–56]. On-line testing and error detection methods, which are suitable for in-field operation have been reported in the literature [10, 57–59].

Apart from defects and malfunctions, droplet contamination can pose a severe threat to the reliable execution of bioassays on a DMF biochip. For example, many biomedical assays involve on-chip transportation of substances containing macromolecules such as DNA, proteins, which may contaminate one or more electrodes [60]. Contamination may have serious erroneous effects on the outcome of the assay running on the chip. Therefore, efficient management and elimination of contamination is very important in order to ensure safe functioning of a biochip [12, 61–63].

7 Conclusions

In this review article, we have summarized the recent advances made in the area of droplet-based LoCs. Solutions to most of these problems require graph-theoretic formulations and optimization techniques. There are many open problems yet to be settled in the area of sample preparation, design, and testing of such chips.

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