# Algorithmic Challenges in Digital Microfluidic Biochips: Protocols, Design, and Test*

Bhargab B. Bhattacharya[1,**], Sudip Roy[2,***], and Sukanta Bhattacharjee[1]

[1] Nanotechnology Research Triangle, Indian Statistical Institute, Kolkata 700108, India
{bhargab,sukanta_r}@isical.ac.in
[2] Dept. of CSE, Indian Institute of Technology Kharagpur, Kharagpur 721302, India
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,

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especially, the immediate point-of-care diagnosis of diseases, enzymatic analysis (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.

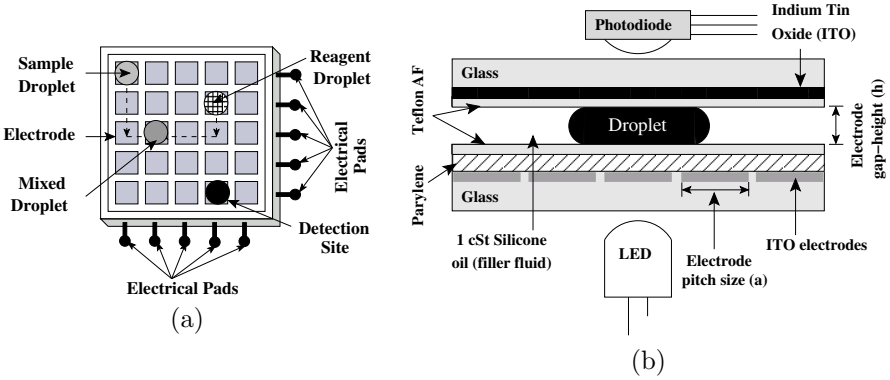


**Fig. 1.** (a) Top view of a DMF biochip and (b) Cross-sectional view of a cell at a detection site [4]

# 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 $\ell$ are non-zero positive integers), $k$-unit volume of one fluid is mixed with $\ell$-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 $\ell$ 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 $\frac{1022+1024}{2.1024}$, 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 input fluids are required in the next step. However, this method works only

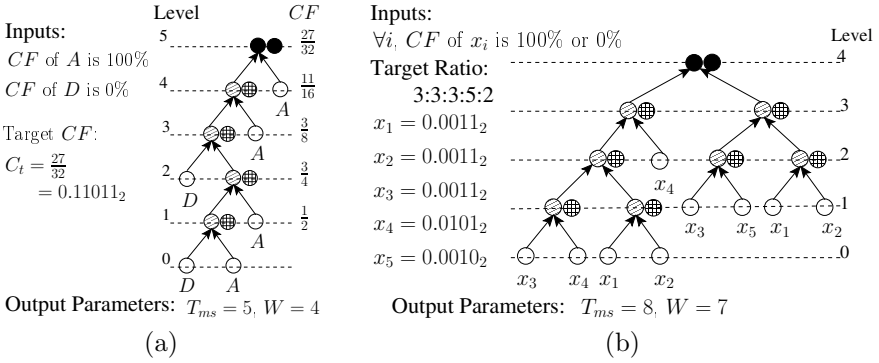


**Fig. 2.** (a) An $(1:1)$-dilution tree of depth $d = 5$ denoting the sequence of five $(1:1)$ mix-split steps for target $CF$ $C_t = 84.375\%$ ($\approx \frac{27}{32} \equiv 0.11011_2$) obtained by *twoWayMix* [15] using fluid $A$ (100% concentration) and buffer (0% concentration). (b) An $(1:1)$-mixing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

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 a 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.

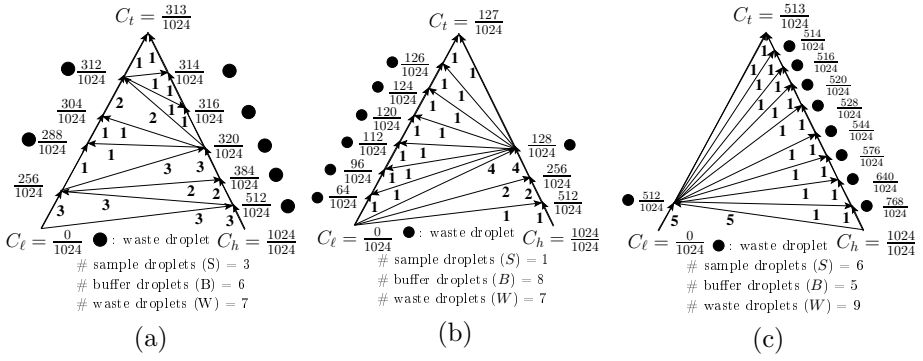


**Fig. 3.** Dilution graphs denoting the sequence of 10 $(k:k)$ mix-split steps to produce target $CF$s (a) $C_t = \frac{313}{1024}$, (b) $C_t = \frac{127}{1024}$ and (c) $C_t = \frac{513}{1024}$, obtained by *DMRW* [34]

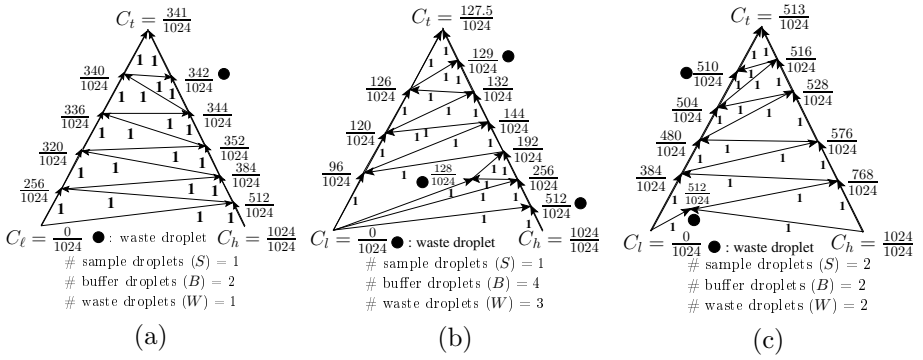


**Fig. 4.** Dilution graphs denoting the sequence of $(k : k)$ mix-split steps to produce target $CF$s (a) $C_t = \frac{341}{1024}$, (b) $C_t = \frac{127}{1024}$ and (c) $C_t = \frac{513}{1024}$, obtained by *IDMA* [34]

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

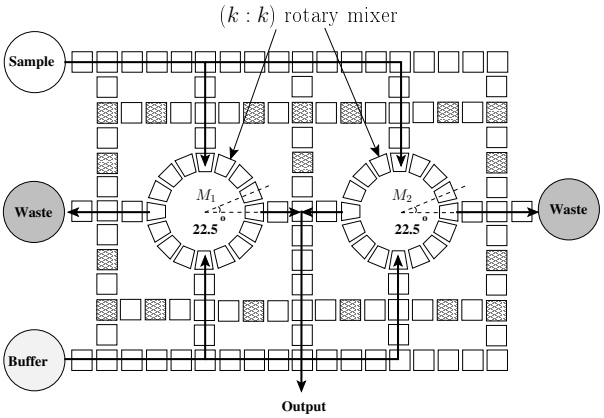


**Fig. 5.** Layout of electrodes with two DMF rotary mixers for $d = 10$ [33]

susceptibility test systems have been developed, which provide results in only few 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 minumim 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

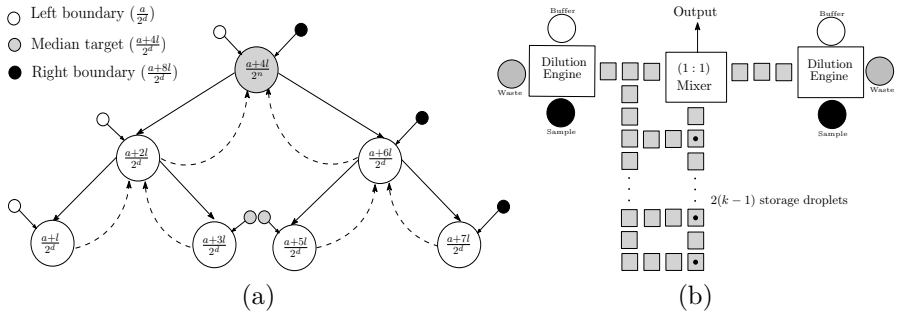


**Fig. 6.** (a) A linear dilution tree generating 9 linear dilutions $\{\frac{a}{2^d}, \frac{a+l}{2^d}, \ldots, \frac{a+8l}{2^d}\}$, where $\frac{a}{2^d}$ and $\frac{a+8l}{2^d}$ are left and right boundary respectively. (b) Architecture layout

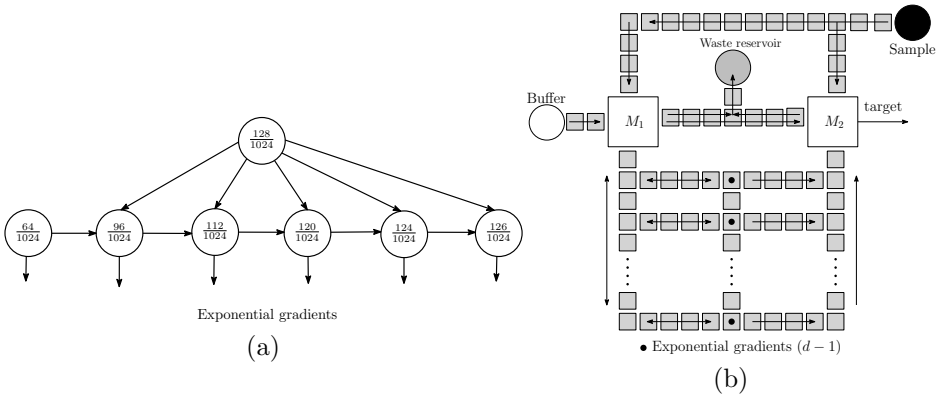


**Fig. 7.** (a) Sequencing graph for an exponential dilution. (b) Architecture layout

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, 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

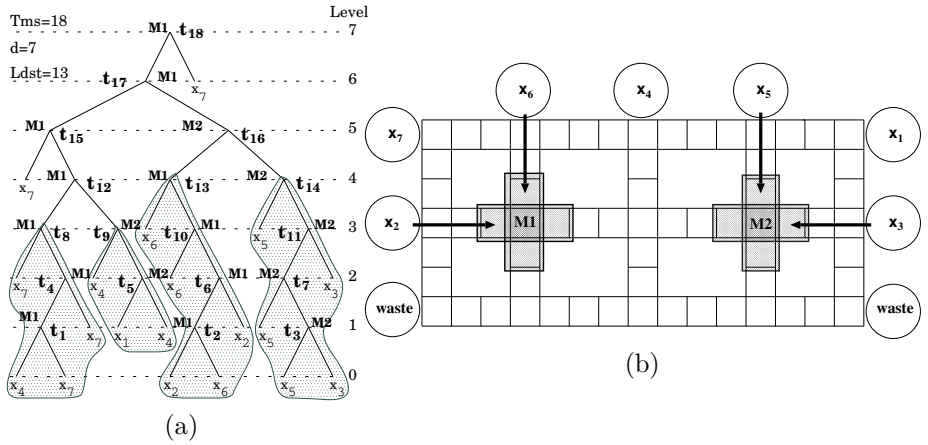


**Fig. 8.** For the example ratio 2 : 3 : 5 : 7 : 11 : 13 : 87, (a) (1 : 1)-mixing tree obtained by *RMA* [44], and (b) droplet routes for the mixing steps of the subtree rooted at $\mathbf{t_{16}}$ of the mixing tree.

mixture preparation. Subsequently, a few other dilution and mixing algorithms were reported with various optimization goals [13, 41, 42, 46–48].

## 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.

## References


[1] Fair, R.B.: Digital Microfluidics: Is a True Lab-on-a-Chip Possible? Microfluid. Nanofluid. 3, 245–281 (2007)

[2] Sista, R., Hua, Z., Thwar, P., Sudarsan, A., Srinivasan, V., Eckhardt, A., Pollack, M., Pamula, V.: Development of a Digital Microfluidic Platform for Point of Care Testing. Lab-on-a-Chip 8(12), 2091–2104 (2008)

[3] Abdelgawad, M., Wheeler, A.R.: The Digital Revolution: A New Paradigm for Microfluidics. Advanced Materials 21, 920–925 (2009)

[4] Chakrabarty, K., Xu, T.: Digital Microfluidic Biochips: Design and Optimization. CRC Press (2010)

[5] Bohringer, K.F.: Modeling and Controlling Parallel Tasks in Droplet-Based Microfluidic Systems. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 25(2), 334–344 (2006)

[6] Yuh, P.H., Yang, C.L., Chang, Y.W.: BioRoute: A Network-Flow-Based Routing Algorithm for the Synthesis of Digital Microfluidic Biochips. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 27(11), 1928–1941 (2008)

[7] Cho, M., Pan, D.Z.: A High-Performance Droplet Routing Algorithm for Digital Microfluidic Biochips. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 27(10), 1714–1724 (2008)

[8] Maftei, E., Pop, P., Madsen, J.: Tabu Search-based Synthesis of Dynamically Reconfigurable Digital Microfluidic Biochips. In: Proc. of the CASES, pp. 195–204 (2009)

[9] Lin, C.C.Y., Chang, Y.W.: ILP-Based Pin-Count Aware Design Methodology for Microfluidic Biochips. In: Proc. of the DAC, pp. 258–263 (2009)

[10] Datta, S., Joshi, B., Ravindran, A., Mukherjee, A.: Efficient Parallel Testing and Diagnosis of Digital Microfluidic Biochips. ACM Journal on Emerging Technologies in Computing Systems 5(2), 1–17 (2009)

[11] Xiao, Z., Young, E.F.: Droplet-Routing-Aware Module Placement for Cross-Referencing Biochips. In: Proc. of the ISPD, pp. 193–199 (2010)

[12] Lin, C.C.Y., Chang, Y.W.: Cross-Contamination Aware Design Methodology for Pin-Constrained Digital Microfluidic Biochips. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 30(6), 817–828 (2011)

[13] Hsieh, Y.L., Ho, T.Y., Chakrabarty, K.: A Reagent-Saving Mixing Algorithm for Preparing Multiple-Target Biochemical Samples Using Digital Microfluidics. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 31(11), 1656–1669 (2012)

[14] Luo, Y., Chakrabarty, K., Ho, T.Y.: Error Recovery in Cyberphysical Digital Microfluidic Biochips. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 32(1), 59–72 (2013)

[15] Thies, W., Urbanski, J.P., Thorsen, T., Amarasinghe, S.: Abstraction Layers for Scalable Microfluidic Biocomputing. Natural Computing 7(2), 255–275 (2008)

[16] OpenWetWare (October 2009), `http://openwetware.org/wiki/Protocols`

[17] BioCoder: A Programming Language for Biology Protocols, Microsoft Research India (December 2009), `http://research.microsoft.com/en-us/um/india/projects/biocoder/`

[18] Bio-Protocols: `http://www.bio-protocol.org/`

[19] Fair, R.B., Srinivasan, V., Ren, H., Paik, P., Pamula, V.K., Pollack, M.G.: Electrowetting-Based On-Chip Sample Processing for Integrated Microfluidics. In: Technical Digest. IEEE International Electron Devices Meeting (IEDM 2003), pp. 32.5.1–32.5.4 (December 2003)
[20] Pollack, M.G., Fair, R.B., Shenderov, A.D.: Electrowetting-based Actuation of Liquid Droplets for Microfluidic Applications. Applied Physics Letters 77, 1725–1726 (2000)
[21] Brassard, D., Malic, L., Normandin, F., Tabrizian, M., Veres, T.: Water-oil Core-shell Droplets for Electrowetting-based Digital Microfluidic Devices. Lab-on-a-Chip 8, 1342–1349 (2008)
[22] Cho, S.K., Moon, H., Kim, C.J.: Creating, Transporting, Cutting, and Merging Liquid Droplets by Electrowetting-based Actuation for Digital Microfluidic Circuits. Journal of Microelectromechanical Systems 12(1), 70–80 (2003)
[23] Fouillet, Y., Jary, D., Chabrol, C., Claustre, P., Peponnet, C.: Digital Microfluidic Design and Optimization of Classic and New Fluidic Functions for Lab on a Chip Systems. Microfluidics and Nanofluidics 4(3), 159–165 (2008)
[24] Paik, P., Pamula, V.K., Fair, R.B.: Rapid Droplet Mixers for Digital Microfluidic Systems. Lab-on-a-Chip 3, 253–259 (2003)
[25] Paik, P., Pamula, V.K., Pollack, M.G., Fair, R.B.: Electrowetting-based Droplet Mixers for Microfluidic Systems. Lab-on-a-Chip 3, 28–33 (2003)
[26] Herold, K.E., Rasooly, A.: Lab-on-a-Chip Technology (vol. 1): Fabrication and Microfluidics. Caister Academic Press (August 2009)
[27] Kim, C., Lee, K., Kim, J.H., Shin, K.S., Lee, K.J., Kim, T.S., Kang, J.Y.: A Serial Dilution Microfluidic Device using a Ladder Network Generating Logarithmic or Linear Concentrations. Lab-on-a-Chip 8(3), 473–479 (2008)
[28] Lee, K., Kim, C., Ahn, B., Panchapakesan, R., Full, A.R., Nordee, L., Kang, J.Y., Oh, K.W.: Generalized Serial Dilution Module for Monotonic and Arbitrary Microfluidic Gradient Generators. Lab-on-a-Chip 9, 709–717 (2009)
[29] Ren, H., Srinivasan, V., Fair, R.B.: Design and Testing of an Interpolating Mixing Architecture for Electrowetting-Based Droplet-On-Chip Chemical Dilution. In: Proc. of the International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS), pp. 619–622 (2003)
[30] Griffith, E.J., Akella, S., Goldberg, M.K.: Performance Characterization of a Reconfigurable Planar-Array Digital Microfluidic System. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 25(2), 345–357 (2006)
[31] Urbanski, J.P., Thies, W., Rhodes, C., Amarasinghe, S., Thorsen, T.: Digital Microfluidics using Soft Lithography. Lab-on-a-Chip 6(1), 96–104 (2006)
[32] Jebrail, M.J., Wheeler, A.R.: Digital Microfluidic Method for Protein Extraction by Precipitation. Journal of Analytical Chemistry 81, 330–335 (2009)
[33] Roy, S., Bhattacharya, B.B., Chakrabarty, K.: Optimization of Dilution and Mixing of Biochemical Samples using Digital Microfluidic Biochips. IEEE Transactions on COMPUTER-AIDED DESIGN of Integrated Circuits and Systems 29(11), 1696–1708 (2010)
[34] Roy, S., Bhattacharya, B.B., Chakrabarty, K.: Waste-Aware Dilution and Mixing of Biochemical Samples with Digital Microfluidic Biochips. In: Proc. of the IEEE/ACM Design, Automation and Test in Europe (DATE) Conference, pp. 1059–1064 (2011)
[35] Cira, N.J., Ho, J.Y., Dueck, M.E., Weibel, D.B.: A self-loading microfluidic device for determining the minimum inhibitory concentration of antibiotics. Lab Chip 12, 1052–1059 (2012)

[36] Sugiura, S., Hattori, K., Kanamori, T.: Microfluidic serial dilution cell-based assay for analyzing drug dose response over a wide concentration range. Analytical Chemistry 82(19), 8278–8282 (2010)
[37] Wang, S., Ji, N., Wang, W., Li, Z.: Effects of non-ideal fabrication on the dilution performance of serially functioned microfluidic concentration gradient generator. In: Nano/Micro Engineered and Molecular Systems (NEMS), pp. 169–172 (2010)
[38] Dertinger, S.K.W., Chiu, D.T., Jeon, N.L., Whitesides, G.M.: Generation of gradients having complex shapes using microfluidic networks. Analytical Chemistry 73(6), 1240–1246 (2001)
[39] Jang, Y.H., Hancock, M.J., Kim, S.B., Selimovic, S., Sim, W.Y., Bae, H., Khademhosseini, A.: An integrated microfluidic device for two-dimensional combinatorial dilution. Lab Chip 11, 3277–3286 (2011)
[40] Bhattacharjee, S., Banerjee, A., Ho, T.Y., Chakrabarty, K., Bhattacharya, B.B.: On producing linear dilution gradient of a sample with a digital microfluidic biochip. In: International Symposium on Electronic System Design, ISED (to appear, 2013)
[41] Roy, S., Bhattacharya, B.B., Ghoshal, S., Chakrabarty, K.: A High-Throughput Dilution Engine for Sample Preparation on Digital Microfluidic Biochips. IET Computers & Digital Techniques (IET-CDT), 9 pages (September 2013)
[42] Huang, J.D., Liu, C.H., Chiang, T.W.: Reactant Minimization during Sample Preparation on Digital Microfluidic Biochips using Skewed Mixing Trees. In: Proc. of IEEE/ACM ICCAD, pp. 377–384 (2012)
[43] Bhattacharjee, S., Banerjee, A., Ho, T.Y., Chakrabarty, K., Bhattacharya, B.B.: Eco-friendly sample preparation with concentration gradient on a digital microfluidic biochip. In: International Conference on Eco-friendly Computing and Communication Systems, ICECCS (to appear, 2013)
[44] Roy, S., Bhattacharya, B.B., Chakrabarti, P.P., Chakrabarty, K.: Layout-Aware Solution Preparation for Biochemical Analysis on a Digital Microfluidic Biochip. In: Proc. of the VLSID, pp. 171–176 (2011)
[45] Roy, S., Chakrabarti, P.P., Kumar, S., Bhattacharya, B.B., Chakrabarty, K.: Routing-Aware Resource Allocation for Mixture Preparation in Digital Microfluidic Biochips. In: IEEE ISVLSI, pp. 1–6 (2013)
[46] Bhattacharjee, S., Banerjee, A., Bhattacharya, B.B.: Sample preparation with multiple dilutions on digital microfluidic biochips. IET Computers & Digital Techniques (IET-CDT), 1–10 (2013)
[47] Mitra, D., Roy, S., Chakrabarty, K., Bhattacharya, B.B.: On-chip sample preparation with multiple dilutions using digital microfluidics. In: Proc. of the IEEE International Symposium on VLSI (ISVLSI), pp. 314–319 (August 2012)
[48] Chiang, T.W., Liu, C.H., Huang, J.D.: Graph-Based Optimal Reactant Minimization for Sample Preparation on Digital Microfluidic Biochips. In: Proc. of the IEEE VLSI-DAT, pp. 1–4 (2013)
[49] Mitra, D.: Studies in High-Throughput and Reliable Assay Operations on Digital Microfluidic Biochips. PhD thesis, Bengal Engineering and Science University, Shibpur, India (2012)
[50] Chakrabarty, K., Su, F.: Digital Microfluidic Biochips: Synthesis, Testing and Reconfiguration Techniques. CRC Press (2007)
[51] Mitra, D., Ghoshal, S., Rahaman, H., Chakrabarty, K., Bhattacharya, B.B.: Test planning in digital microfluidic biochips using improved eulerization techniques and the Chinese postman problem. In: IEEE Asian Test Symposium (ATS), pp. 111–116 (2010)

[52] Mitra, D., Ghoshal, S., Rahaman, H., Chakrabarty, K., Bhattacharya, B.B.: Test planning in digital microfluidic biochips using efficient eulerization techniques. Journal of Electronic Testing: Theory and Applications 27(5), 657–671 (2011)
[53] Su, F., Hwang, W., Mukherjee, A., Chakrabarty, K.: Testing and diagnosis of realistic defects in digital microfluidic biochips. Journal of Electronic Testing: Theory and Applications 23(2-3), 219–233 (2007)
[54] Xu, T., Chakrabarty, K.: Fault modeling and functional test methods for digital microfluidic biochips. IEEE Transactions on Biomedical Circuits and Systems 3(4), 241–253 (2009)
[55] Xu, T., Chakrabarty, K.: Functional testing of digital microfluidic biochips. In: International Test Conference (ITC), pp. 1–10 (2007)
[56] Mitra, D., Ghoshal, S., Rahaman, H., Bhattacharya, B.B., Majumder, D.D., Chakrabarty, K.: Accelerated functional testing of digital microfluidic biochips. In: IEEE Asian Test Symposium (ATS), pp. 295–300 (2008)
[57] Zhao, Y., Chakrabarty, K.: On-line testing of lab-on-chip using digital microfluidic compactors. In: IEEE International On-Line Testing Symposium, pp. 213–218 (2008)
[58] Zhao, Y., Chakrabarty, K.: Pin-count-aware online testing of digital microfluidic biochips. In: IEEE VLSI Test Symposium (VTS), pp. 111–116 (2010)
[59] Mitra, D., Ghoshal, S., Rahaman, H., Chakrabarty, K., Bhattacharya, B.B.: Online error detection in digital microfluidic biochips. In: IEEE Asian Test Symposium (ATS), pp. 332–337 (2012)
[60] Fair, R.B., Khlystov, A., Tailor, T.D., Ivanov, V., Evans, R.D., Griffin, P.B., Srinivasan, V., Pamula, V.K., Pollack, M.G., Zhou, J.: Chemical and Biological Applications of Digital-Microfluidic Devices. IEEE Design & Test of Computers 24(1), 10–24 (2007)
[61] Zhao, Y., Chakrabarty, K.: Cross-contamination avoidance for droplet routing in digital microfluidic biochips. In: IEEE/ACM Design, Automation and Test in Europe (DATE), pp. 1290–1295 (2009)
[62] Mitra, D., Ghoshal, S., Rahaman, H., Chakrabarty, K., Bhattacharya, B.B.: On residue removal in digital microfluidic biochips. In: Great Lakes Symposium on VLSI (GLSVLSI), pp. 391–394 (2011)
[63] Mitra, D., Ghoshal, S., Rahaman, H., Chakrabarty, K., Bhattacharya, B.B.: Automated path planning for washing in digital microfluidic biochips. In: IEEE International Conference on Automation Science & Engineering (CASE), pp. 115–120 (2012)