Reactant and Waste Minimization in Multitarget Sample Preparation on Digital Microfluidic Biochips

Juinn-Dar Huang, *Member, IEEE,* Chia-Hung Liu, *Student Member, IEEE,* and Huei-Shan Lin

***Abstract*—Sample preparation is one of essential processes in biochemical reactions. Raw reactants are diluted in this process to achieve given target concentrations. A bioassay may require several different target concentrations of a reactant. Both the dilution operation count and the reactant usage can be minimized if multiple target concentrations are considered simultaneously during sample preparation. Hence, in this paper, we propose a multitarget sample preparation algorithm that extensively exploits the ideas of waste recycling and intermediate droplet sharing to reduce both reactant usage and waste amount for digital microfluidic biochips. Experimental results show that our waste recycling algorithm can reduce the waste and operation count by 48% and 37%, respectively, as compared to an existing state-of-the-art multitarget sample preparation method if the number of target concentrations is ten. The reduction can be up to 97% and 73% when the number of target concentrations goes even higher.**

***Index Terms*—Biochip, digital microfluidic biochip (DMFB), dilution, multitarget sample preparation, reactant minimization, waste minimization.**

I. Introduction

LAB-ON-A-CHIP (LoC) is one of the emerging applications in bioelectronics. An LoC is an analysis system that implements various biochemical protocols or assays in a small chip, such as sample preparation, injection, separation, and detection [1]. Compared with conventional biochemical analysis systems, LoCs offer many advantages like portability, reagent volume reduction, automation, mass production, fast analysis, high throughput, and low power consumption [2]. A new type of LoC, digital microfluidic biochip (DMFB), has been developed in recent years. A DMFB carries out various bioassays by precisely controlling small volume fluidic droplets containing biochemical samples or reagents. The electrowetting-on-dielectrics (EWOD) effect, an electrostatic actuation method, is utilized to dispense, transport, split, merge, and mix droplets on DMFBs through a proper voltage control over on-chip electrodes [3]–[6]. A biochemical assay can be completed via a series of basic droplet operations mentioned above. Since no prefabricated channels and pumps are required, the applicability of DMFB is thus greatly enhanced [3].

Recently, numerous on-chip laboratory procedures, such as immunoassay, protein crystallization, and DNA sequencing, have all been successfully demonstrated on DMFBs [7]. Because the demand continuously grows, the design complexity of DMFB is accordingly increased. Therefore, related design automation tools are indispensable for reducing the manual effort, speeding up the design process, and improving the design quality. In the past few years, a number of studies have been conducted to address design automation issues of DMFB, such as synthesis, placement, routing, control pin assignment, and testing [8]–[19]. Undoubtedly, the DMFB-related design automation is one of the emerging research topics nowadays.

Sample preparation is an essential step in biochemical reactions. Since an assay may require a reactant (sample or reagent) in a definite concentration, a raw reactant must be diluted to that specific concentration, called target concentration, during sample preparation [20]–[22]. Hence, how to optimize the dilution process is an issue worth studying. In general, there are two fundamental minimization goals for a dilution process: 1) the usage of valuable reactants, and 2) the number of dilution operations. A valuable reactant can be a limited amount of sample (e.g., infant's blood) or a very expensive reagent. On the other hand, the number of dilution operations roughly decides the sample preparation time, and thus should be minimized as well.

Recently, several research works addressing the sample preparation problem on DMFBs have been proposed [23]–[33]. Most of the previous techniques concentrate on the problem of single-target sample preparation, which considers merely one target concentration at a time. In case multiple target concentrations are demanded, every target concentration is still produced by an individual dilution process exclusively. That is, multiple target concentrations are actually produced one by one and the whole sample preparation process therefore becomes time-consuming. Moreover, these methods do not consider reactant sharing among different targets, which leads to higher reactant usage and more waste.

The first approach focusing on the problem of concurrent preparation for multiple target concentrations was presented in [28]. It is named intermediate droplet sharing algorithm (IDSA), which minimizes the number of required intermediate concentrations in dilution for waste reduction. It is generally assumed that less waste implies faster sample preparation. Nevertheless, minimizing the number of intermediate

Manuscript received August 16, 2012; revised February 25, 2013; accepted April 14, 2013. Date of current version September 16, 2013. This paper was recommended by Associate Editor Y. Chen.

The authors are with Department of Electronics Engineering, National Chiao Tung University, Hsinchu 30010, Taiwan (e-mail: jdhuang@mail.nctu.edu.tw; terryliu@adar.ee.nctu.edu.tw; hslin@adar.ee.nctu.edu.tw).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TCAD.2013.2263035

concentrations does not necessarily reduce the number of waste droplets. Furthermore, in our opinion, minimizing the usage of valuable reactant is as important as waste reduction. Therefore, a better alternative that can minimize reactant and waste simultaneously in multitarget sample preparation should be further developed.

In this paper, we propose a waste recycling algorithm (WARA) for multitarget sample preparation on DMFBs. WARA first utilizes a single-target sample preparation algorithm [32] to create a reactant-minimized mixing tree for each target concentration. Then, it tries to maximize droplet sharing and waste recycling among those trees for reactant and waste minimization. Experimental results demonstrate that WARA reduces the amount of reactant, waste, and dilution operations by 10%, 17%, and 16%, respectively, on average as compared to a reactant-minimized single-target-at-a-time preparation method even when the number of targets is merely three. When compared with IDSA, WARA still reduces the amount of waste and operations by 48% and 37%, respectively, on average if the number of targets is ten.[1] The reduction can be up to 97% and 73% as the number of target concentrations grows to 100. The results suggest that WARA should be a better solution for multitarget sample preparation on DMFBs

The rest of this paper is organized as follows. Section II describes the sample preparation process and different mixing models. Section III briefly introduces several existing techniques and elaborates on the IDSA approach. Section IV shows the motivation and the problem formulation of this paper. Section V presents our multitarget sample preparation algorithm WARA in detail. Experimental results are then reported and discussed in Section VI. Finally, Section VII concludes this paper.

II. Sample Preparation

A. Dilution Procedures

The goal of sample preparation is to prepare one or more specified target concentrations (C_t) through a series of dilution operations. On biochips, both linear dilution and serial dilution procedures are commonly used [20]–[21]. However, the linear dilution is not that suitable as the serial one on DMFBs because reactants are dispensed as discrete droplets instead of continuous flows. The serial dilution process dilutes a reactant droplet repeatedly using a fixed mixing ratio, like 1:1. For example, if C_t is set to 25%, the serial dilution method first dilutes a reactant droplet with a buffer droplet to produce a mixture with the concentration of 50%. Then that mixture, or intermediate droplet, is again diluted with a buffer droplet to achieve the target concentration (i.e., 25%). In the above case, two serial dilution operations are counted. Note that a whole serial dilution process may need numerous individual dilution operations [21]. Therefore, a smaller dilution operation count usually implies a faster sample preparation process.

[1]Since IDSA reported its results by means of bar charts, we have done our best to compare our work with IDSA as accurately as possible.

B. Mixing Models

Different mixing models are available on various DMFB architectures. In previous studies, three mixing models are commonly used. Suppose that the ratio between two substances for mixing is $(x: y)$, then those three models can be expressed as: 1) $x = y = 1$; 2) $x = y \neq 1$; and 3) $x \neq y$. Previous approaches [26]–[28] adopt the second mixing model by means of a specially-designed rotary mixer to produce multiple droplets with the same desired concentration simultaneously. However, the rotary mixer is not a vital component and occupies significant chip area. Therefore, in most general DMFB designs, only the first mixing model can be easily implemented through the use of linear or array mixers [4]. Consequently, we adopt that mixing model, namely, (1:1) mixing model, in this paper, just as the previous works [25], [29], [30], [32], and [33] do. As well, the number of (1:1) dilution operations is then used to estimate the sample preparation time.

However, no matter whether the first or the second mixing model is adopted, a difference between the target concentration and the value that can be actually achieved may exist. The error is inevitable if the denominator of the target concentration is not a power of two or even not a rational number, like 3 or $\sqrt{3}$. However, this error can be reduced down to an acceptable value if the precision level is high enough [25]–[26]. A precision level n indicates that n fractional bits are used to represent the target concentration and the error can thus be limited to $1/2^{n+1}$. Users can determine a proper precision level to keep this error tolerable to their applications.

C. Exponential and Interpolated Dilution

Under the (1:1) mixing model, a dilution operation first mixes two source droplets into a mixture and then splits it into two resultant droplets [21]. Hence the two resultant droplets have the same concentration value (CV). The relation between these droplets can be expressed as

$$C_r = \frac{C_1 + C_2}{2} \tag{1}$$

where C_1 and C_2 represent the CVs of two source droplets and C_r is the CV of the resultant droplets. Dilution operations can be further classified into two types: exponential dilution and interpolated dilution [26]. For an exponential dilution operation, one of its source droplets is buffer. If a source droplet with $\mathrm{CV} = C$ is diluted with buffer, the CV of the resultant droplet is $C/2$. Keep diluting the resultant droplet with buffer and repeat the same process by m–1 more times, the CV of the final resultant droplet eventually becomes $C/2^m$. In this paper, a concentration value C is called a prime concentration value (PCV) if C is equal to 1 or can be produced exclusively through a series of exponential dilutions starting from a raw reactant droplet (i.e., $\mathrm{CV} = 1$), for example, 1/16. That is, a PCV contains only one bit of '1' in its binary representation. On the other hand, for an interpolated dilution operation, none of its source droplets is buffer. Note that both exponential and interpolated dilution operations are necessary to achieve an arbitrary target concentration value.

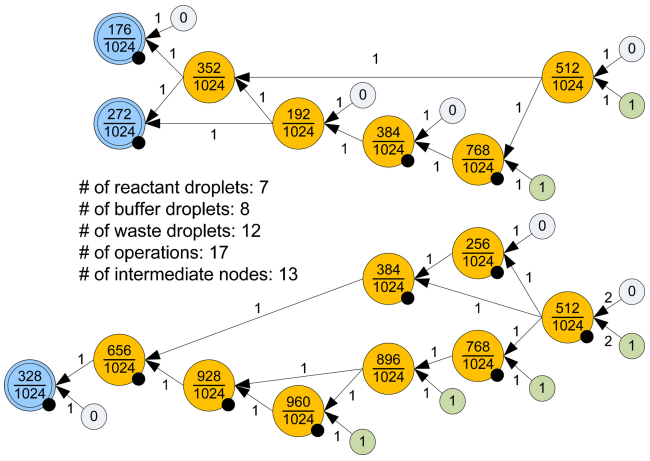


Fig. 1. Dilution graph.

III. Previous Works

The first method that addresses the dilution control on DMFBs was proposed in [23]. It focuses on the single-target sample preparation problem, and adopts a binary search strategy to guide the dilution process. Other related previous works include the bit-scanning (BS) method [25], the algorithm for dilution and mixing with reduced wastage (DMRW) [26], the improved dilution/mixing algorithm (IDMA) [27], IDSA [28], the ratioed mixing algorithm (RMA) [29], the De Bruijn graph-based multitarget preparation scheme (DBG) [30], the reactant minimization algorithm (REMIA) [32], and the graph-based optimal reactant minimization algorithm [33]. Most of previous approaches address the single-target sample preparation problem. If multiple targets are required, they must be produced one by one. So far, IDSA is the first work that focuses on the multitarget sample preparation problem. The primary objective of IDSA is to minimize the waste. More precisely, IDSA actually tries to minimize the number of intermediate concentrations during sample preparation. The authors of IDSA assume that the waste amount and operation count can also be reduced accordingly. The other existing technique for multitarget sample preparation is DBG [30]. It concentrates on dilution step minimization and ensures no storage unit is required. Since DBG is not developed for reactant or waste minimization, we only elaborate IDSA in the rest part of this section.

IDSA is a two-phased algorithm. In the first phase, an initial dilution graph is generated to guide the dilution process, as shown in Fig. 1. In a dilution graph, a node with in-degree of 0 is associated with either raw reactant or buffer; a node with out-degree of 0 is associated with a target CV; every other node contains an intermediate CV; the number beside an edge indicates the count of required droplets; a black dot beside a node implies a waste droplet.

There may exist multiple source pairs that can lead to a same target CV after dilution, for example, $(1/2, 1/4)$ and $(0, 3/4)$ for 3/8. These source pairs are named preceding pairs of a target CV. IDSA enumerates all preceding pairs for every CV and then builds a table to store them via dynamic programming, which is very time-consuming; the time complexity is about $O(2^{2n})$, where n is the precision level. IDSA traces all feasible preceding pairs recursively starting from every target CV, selects the intermediate concentrations, and thus constructs the initial dilution graph.

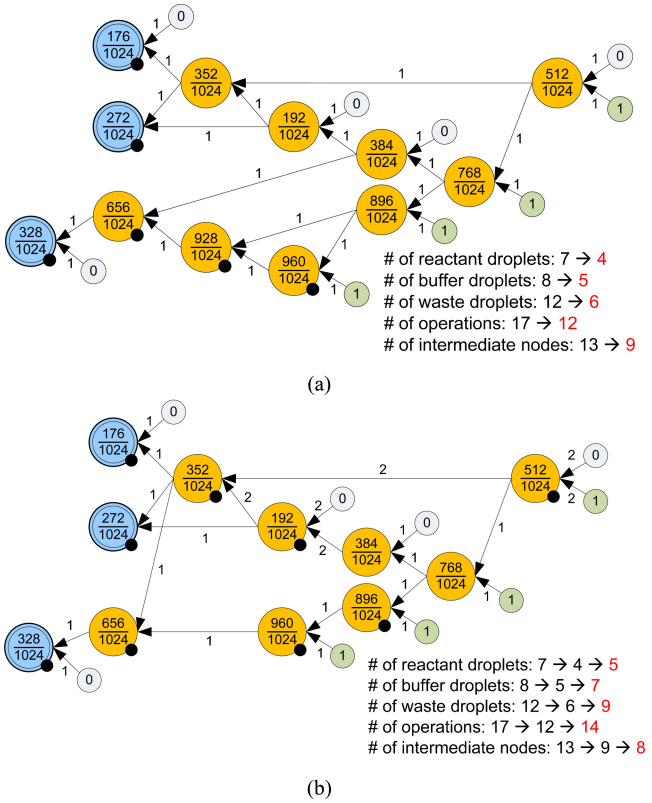


Fig. 2. Dilution graph after (a) sharing and (b) replacement.

The second phase of IDSA tries to eliminate the number of intermediate nodes. Two strategies are adopted in this phase: node sharing and node replacement. For example, IDSA shares the common nodes of CV = 384/1024 and 768/1024 in Fig. 1, and the result after sharing is shown in Fig. 2(a). IDSA further reduces the number of intermediate nodes through node replacement. For instance, the preceding pair (352/1024, 960/1024) replaces (384/1024, 928/1024) for the node of CV = 656/1024, and the node of CV = 928/1024 can thus be eliminated, as illustrated in Fig. 2(b).

However, Fig. 2(b) demonstrates that the node replacement technique used in IDSA is not always able to reduce the number of waste droplets. Moreover, it may even increase the operation count and reactant usage, also indicated in Fig. 2(b). Therefore, a more appropriate optimization objective instead of node minimization should be considered. As well, IDSA is an exponential algorithm and thus might not be applicable as the precision level goes higher.

IV. Motivation

A. Tree-Based Approaches

Unlike approaches [26]–[28] and [33] based on the dilution graph, all of BS [25], RMA [29], DBG [30], and REMIA [32] adopt another strategy, named mixing tree. Two mixing

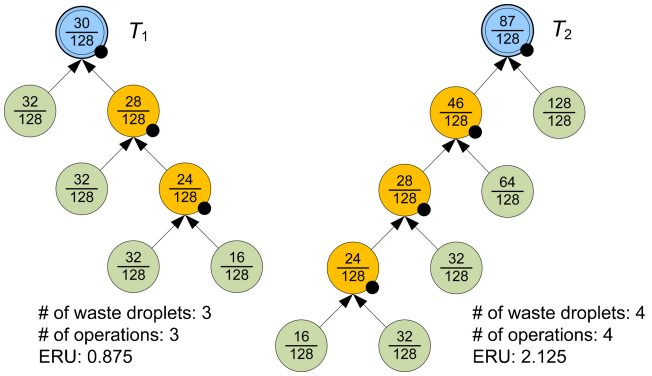


Fig. 3. Mixing trees for $C_t = 30/128$ and 87/128.

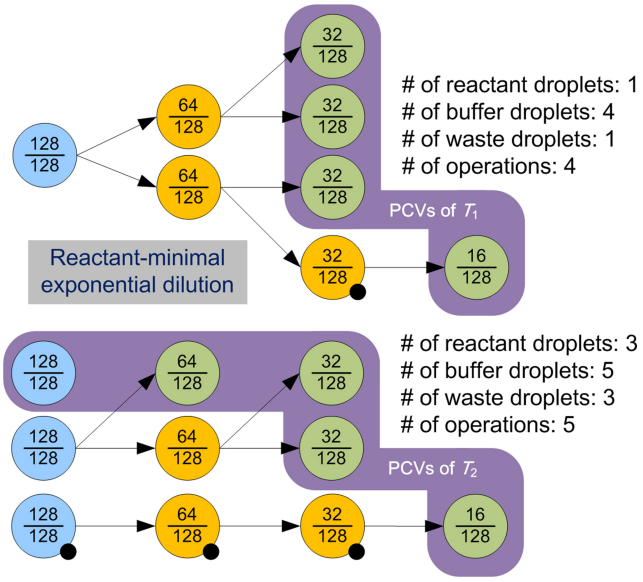


Fig. 4. Reactant-minimal exponential dilution processes for producing leaf nodes of T_1 and T_2 shown in Fig. 3.

trees for $C_t = 30/128$ and 87/128 are shown in Fig. 3. A node is a PCV node if its value is a PCV. A node with in-degree of 0 (i.e., leaf node) in a mixing tree must be a PCV node. Furthermore, a mixing tree must be a full binary tree, in which every branch node has exactly two children since a dilution operation always requires two source droplets. Nevertheless, only one of the two resultant droplets is required for succeeding operations. As a result, every branch node implies a waste droplet. The relation between the number of dilution operations, branch nodes, and waste droplets in a mixing tree T can be expressed as

$$\#\text{branch\_node}(T) = \#\text{operation}(T) = \#\text{waste}(T). \quad (2)$$

The overall reactant consumption for a mixing tree remains unknown until the exact process about how the leaf nodes are produced is determined. Nevertheless, the minimal reactant usage can be estimated by the essential reactant usage (ERU), which is equal to the sum of the CVs of all leaf nodes [32]. The minimal number of required reactant droplets can thus be calculated by further rounding up the ERU. That is, let CV(v) denote the CV of a node v, the relation between the minimal number of reactant droplets and the ERU can be described as

$$\begin{aligned} \#\text{reactant}(T) &\geq \min \#\_\text{reactant}(T) \\ &= \lceil \text{ERU}(T) \rceil = \left\lceil \textstyle\sum_{v \in leaf(T)} \text{CV}(v) \right\rceil. \end{aligned} \quad (3)$$

In this paper, we utilize a reactant-minimal exponential dilution process that guarantees to produce all required leaf nodes of a mixing tree T with minimal reactant usage, i.e., #reactant(T) = min#_reactant(T) [32]. The process is very similar to the well-known Huffman encoding algorithm [34]. Fig. 4 demonstrates the processes for producing leaf nodes of the two mixing trees, T_1 and T_2, originally illustrated in Fig. 3, where #reactant(T_1) = [ERU(T_1)] = 1 and #reactant(T_2) = [ERU(T_2)] = 3.

There may exist several feasible mixing trees for a given target CV. In this paper, we adopt REMIA [32], which is the best known method for reactant minimization in single-target sample preparation, for mixing tree generation. REMIA utilizes an efficient top-down decomposition strategy to build a skewed mixing tree (SMT) with extremely low ERU for a given target CV. In the multitarget preparation problem, the leaf nodes of all SMTs can be produced jointly through an optimal unified exponential dilution process. The overall reactant usage can thus be further reduced because

$$\left\lceil \sum_T \text{ERU}(T) \right\rceil \leq \sum_T \lceil \text{ERU}(T) \rceil. \quad (4)$$

For example, the two individual reactant-minimal exponential dilution processes shown in Fig. 4 can be replaced by one optimal unified dilution process indicated in Fig. 5, and the total of required reactant droplets is thus reduced from four to three. The unified dilution process also decreases the amount of buffer, waste, and operations. Moreover, according to the property of the ceiling function, it guarantees that the overall wasted reactant is less than one droplet, as shown below

$$\left\lceil \sum_T \text{ERU}(T) \right\rceil - \sum_T \text{ERU}(T) < 1. \quad (5)$$

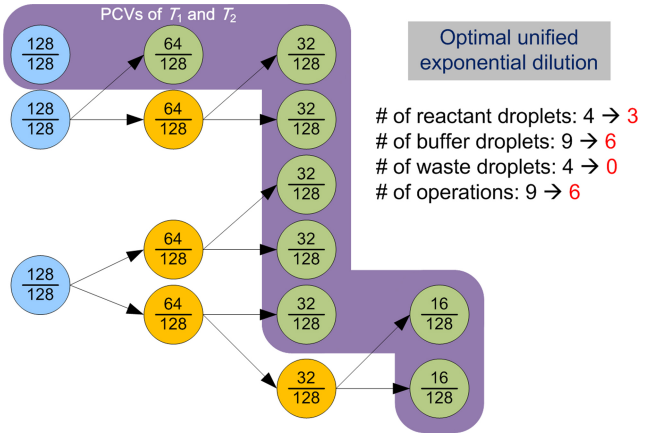


Fig. 5. Optimal unified exponential dilution process for producing all the leaf nodes shown in Fig. 3.

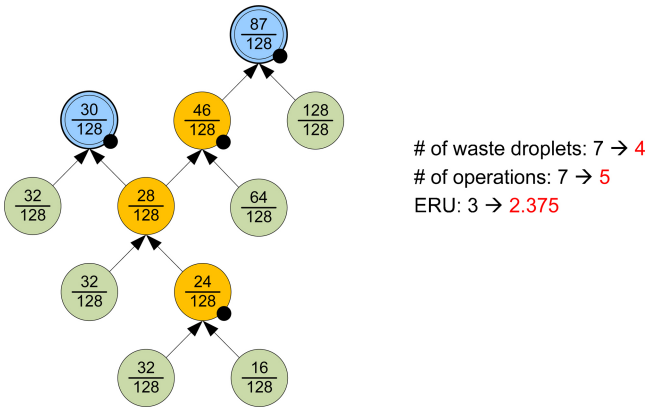


Fig. 6. Result after droplet sharing on the two mixing trees shown in Fig. 3.

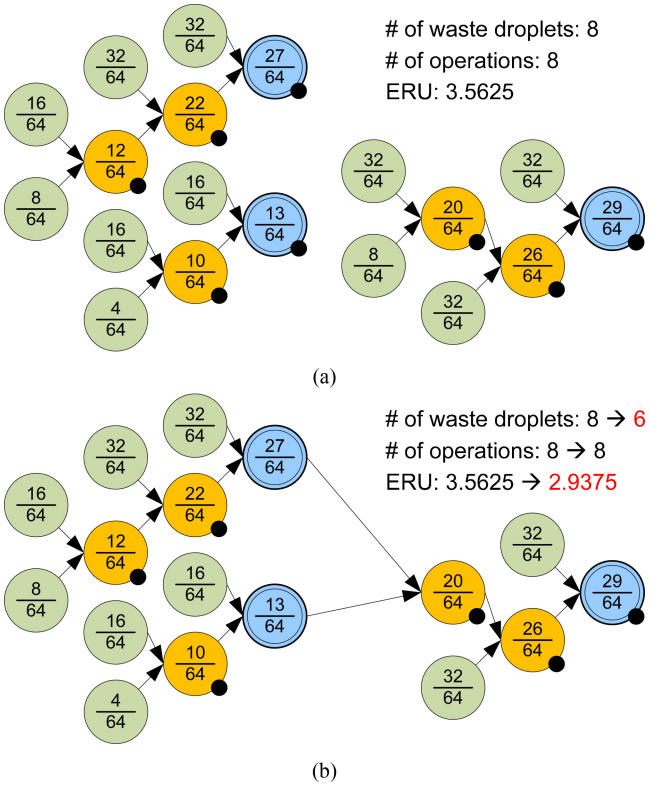


Fig. 7. (a) Three SMTs for C_t = 13/64, 27/64 and 29/64. (b) Result after replacing the branch node with CV = 20/64.

B. Motivation

Every branch node in Fig. 3 implies a waste droplet. However, those waste droplets can actually be saved if one waste droplet produced in a mixing tree is useful in the other mixing tree while preparing multiple targets. For example, if the two mixing trees shown in Fig. 3 share the same branch node with CV = 28/128, the overall ERU is reduced from three to 2.375 and the number of waste droplets is decreased from seven to four, as depicted in Fig. 6. Hence, sharing branch nodes between trees can minimize both the reactant usage and the waste amount.

In addition to the strategy of sharing common branch nodes, we found that recycling waste droplets can also improve the

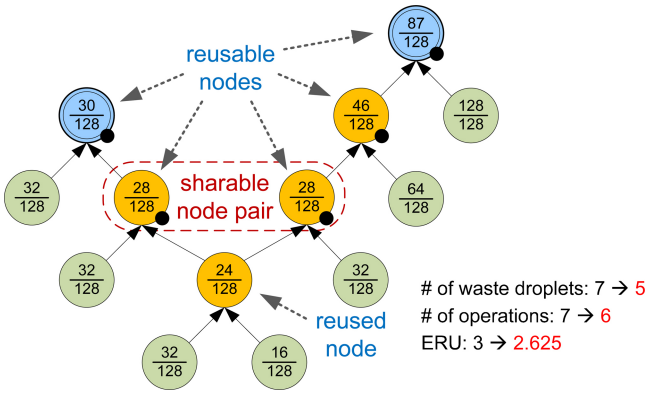


Fig. 8. Intermediate result of droplet sharing for the case shown in Fig. 3.

DROPLET-SHARING(F)
// F is a forest containing a set of mixing trees
// every branch node in F is initially labeled as a reusable node
1. **while** (there is a sharable node pair)
2. identify a sharable node pair (x, y) with the largest nzb
3. for y's fanout node z, substitute y with x as z's fanin node
4. remove the subtree rooted at y
5. label x as a reused node
6. **return** the resultant dilution graph G

Fig. 9. Pseudo code of droplet sharing.

outcome. For example, Fig. 7(a) illustrates three SMTs for C_t = 13/64, 27/64, and 29/64. Initially, there is one waste droplet for the nodes with CV = 13/64 and 27/64 each. Then, a node with CV = 20/64 can be produced by recycling these two waste droplets and applying interpolated dilution. The newly generated droplet can thus be used to replace the original one with the same CV as depicted in Fig. 7(b). It is obvious that the method of turning waste droplets into useful ones can effectively reduce both the waste and the reactant usage. This recycling-then-replacing optimization strategy is named droplet replacement.

In this paper, the multitarget sample preparation problem is formally formulated as follows. Given a set of target concentration values, determine a dilution process under the (1:1) mixing model such that the reactant usage and the waste amount can both be minimized. The proposed algorithm WARA, which extensively exploits both droplet sharing and droplet replacement, is detailed in the next section.

V. Proposed Algorithm

A. Algorithm Overview

WARA divides an interpolated dilution process into three consecutive phases: mixing tree generation, droplet sharing, and droplet replacement. In the first phase, WARA builds a forest F that contains a set of SMTs using REMIA; each SMT is associated with a given target CV. In the second phase, WARA performs droplet (i.e., node) sharing within F for preliminary waste and reactant minimization. It further refines the dilution process via droplet replacement in the third phase. Finally, the optimal unified exponential dilution

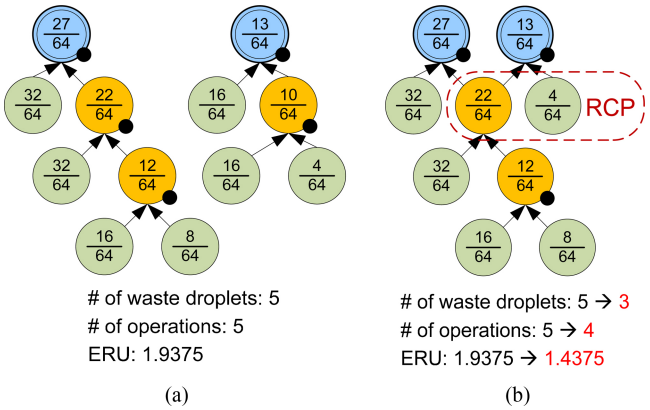


Fig. 10. (a) Two SMTs for C_t = 27/64 and 13/64. (b) Result after droplet replacement.

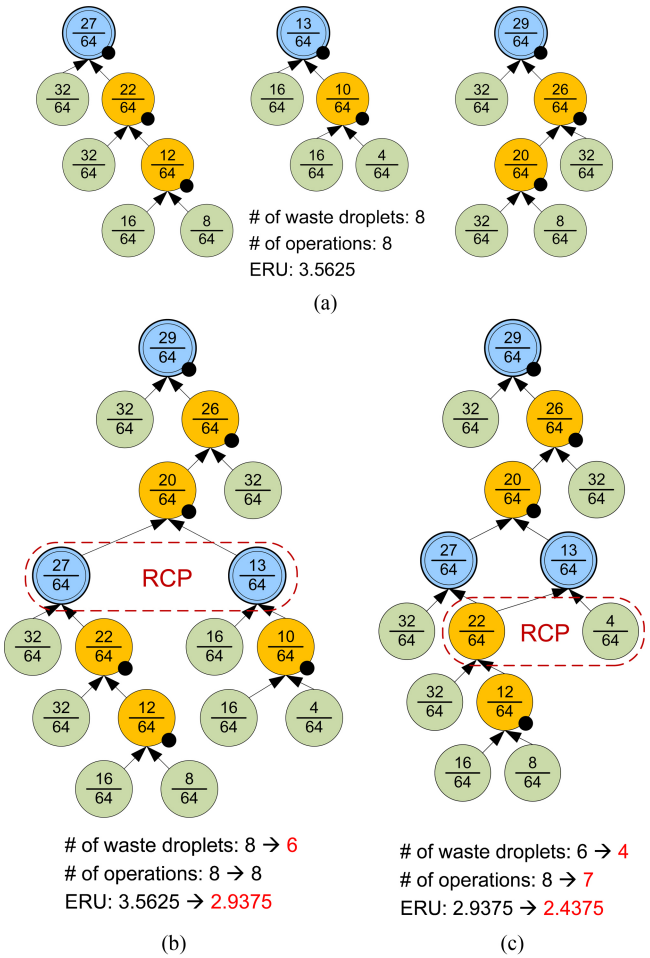


Fig. 11. Example of droplet replacement. (a) Initial graph G. (b) After the first replacement. (c) After the second replacement.

process, as described in Section IV-A, is followed to produce all required PCV nodes. In the rest of this section, we elaborate how droplet sharing and droplet replacement work exactly.

B. Droplet Sharing

A branch node associated with a waste droplet is referred to as a reusable node. For example, in Fig. 3, the nodes with CV = 24/128, 28/128, 30/128, 46/128, and 87/128 are all

DROPLET-REPLACEMENT(G)
// G is a dilution graph right after droplet sharing
1. **while** (there is a replacement candidate pair (RCP))
2. find all RCPs
3. calculate $ac(v)$ for every node v in RCPs
4. calculate $gain(p)$ and $uniq(p)$ for every RCP p
5. sort all RCPs – primary: $gain$, secondary: $uniq$
6. identify RCP $q(x, y)$ with the highest precedence
7. apply droplet replacement using q; update G accordingly
8. label x and y as reused nodes if they are not PCV nodes
9. **return** the resultant dilution graph G'

Fig. 12. Pseudo code of droplet replacement.

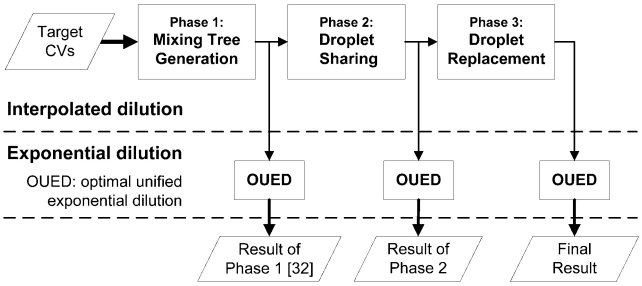


Fig. 13. Experimental flow.

reusable nodes. The waste droplet of a reusable node may be recycled by other dilution operations by either droplet sharing or droplet replacement. In contrast, a branch node with no waste droplet is referred to as a reused node. For instance, the node with CV = 28/128 in Fig. 6 is a reused node. Also note that every branch node is initially a reusable one before droplet sharing starts.

The procedure of droplet sharing begins with identifying sharable node pairs. A node pair (x, y) is sharable if both nodes are reusable and $CV(x) = CV(y)$. Assume (x, y) is a sharable node pair and node z represents the dilution operation taking y as one of its source droplets, then z can take x as its source droplet instead since $CV(x) = CV(y)$. As a consequence, x becomes a reused node because it is utilized at two different places. In the meantime, the nodes in the subtree rooted at y can thus be safely removed. It is obvious that droplet sharing can effectively minimize both the reactant usage as well as the waste amount. Moreover, the operation count can also be reduced due to a smaller number of branch nodes.

When there exist multiple sharable node pairs at the same time, the order of pair selection is irrelevant; that is, the outcome at the end of droplet sharing is eventually identical no matter what the order is. For example, there are two sharable node pairs in Fig. 3, one is with CV = 24/128 and the other is with CV = 28/128. Fig. 8 demonstrates the intermediate result if the node pair with CV = 24/128 is selected for sharing first. Since there is still one sharable node pair with CV = 28/128 left in Fig. 8, the second run of droplet sharing is followed and the final result is given in Fig. 6. However, if the node pair with CV = 28/128 instead of 24/128 is selected first, the result of the first run is still the one shown in Fig. 6. Therefore, it is definite that the order of pair selection has no influence on the outcome of droplet sharing.

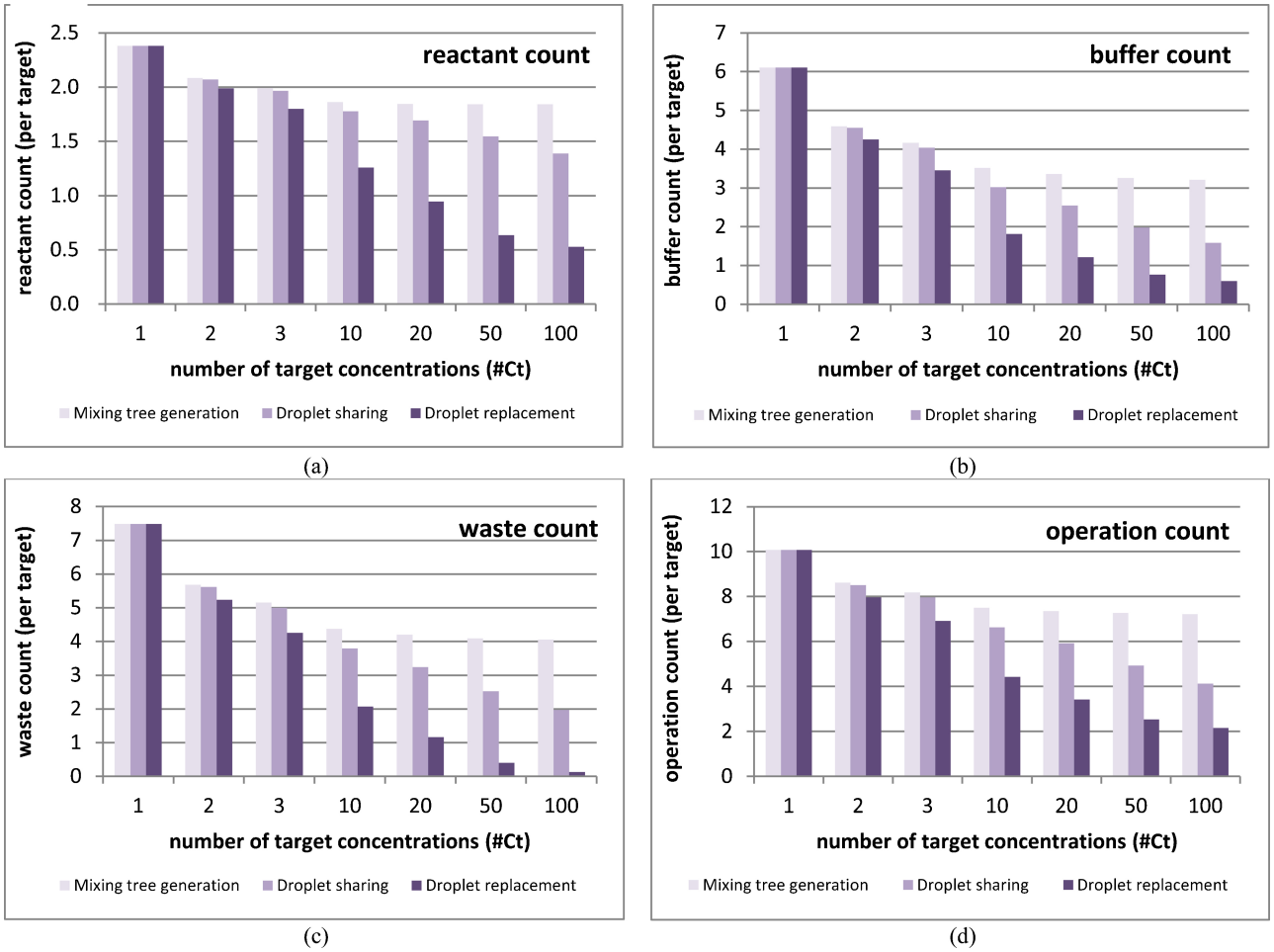


Fig. 14. (a) Reactant, (b) buffer, (c) waste, and (d) operation count per target for various $\#C_t$.

Nevertheless, the previous example suggests that the order of pair selection does impact the runtime efficiency of droplet sharing though it does not affect the final result. It also points out a trend—the farther a pair is from PCV nodes, the higher priority it should own since a farther node pair p can potentially eliminate the need of sharing those node pairs resided in the fanin cone of p, which improves the runtime efficiency and is exactly what Figs. 6 and 8 jointly demonstrate. We further discover that for a node v the number of nonzero bits in the binary representation of CV(v), referred to as $nzb(v)$, can help determine the order of pair selection. If x and y are two fanin nodes of node z, then $nzb(z)$ is equal to the sum of $nzb(x)$ and $nzb(y)$ due to the inherent nature of SMT. That is, $nzb(x)$ must be smaller than $nzb(z)$ if x is a fanin of z. Therefore, when there exist two or more sharable node pairs, the one with the largest nzb should be first selected for sharing. The process of droplet sharing is not terminated until no sharable node pair can be found. Fig. 9 outlines the proposed droplet sharing flow.

C. Droplet Replacement

After droplet sharing, unpaired reusable nodes may still exist. Therefore, we further propose a method that can keep recycling those remaining reusable nodes. Assume x and y are two reusable nodes and z is a node residing at neither x's fanin cone nor y's fanin cone; then the pair (x, y) is called a replacement candidate pair (RCP) of z if CV(z) is exactly equal to the half of the sum of CV(x) and CV(y). That is, the resultant droplet w obtained by mixing the two waste droplets of x and y can be utilized to replace z. If the replacement does take place, z and its exclusive predecessor nodes can thus be safely removed. Hence, it is apparent that droplet replacement can also reduce the reactant usage as well as the waste amount simultaneously.

Unlike droplet sharing, the outcome of droplet replacement is order dependent. For example, for three reusable nodes x, y, and z, there may exist three feasible RCPs (x, y), (y, z), and (x, z) at most. Nevertheless, selecting (x, y) for droplet replacement would make (y, z) and (x, z) no longer feasible RCPs. It implies that a considerate strategy for RCP ordering is demanded. Since the reactant minimization is the primary optimization objective, it should be a good idea to sort RCPs by total ERU saving. That is, assume G/G' are the graphs before/after an RCP p is selected for droplet replacement, the gain of p is formulated as

$$gain(p) = \mathrm{ERU}(G) - \mathrm{ERU}(G'). \tag{6}$$

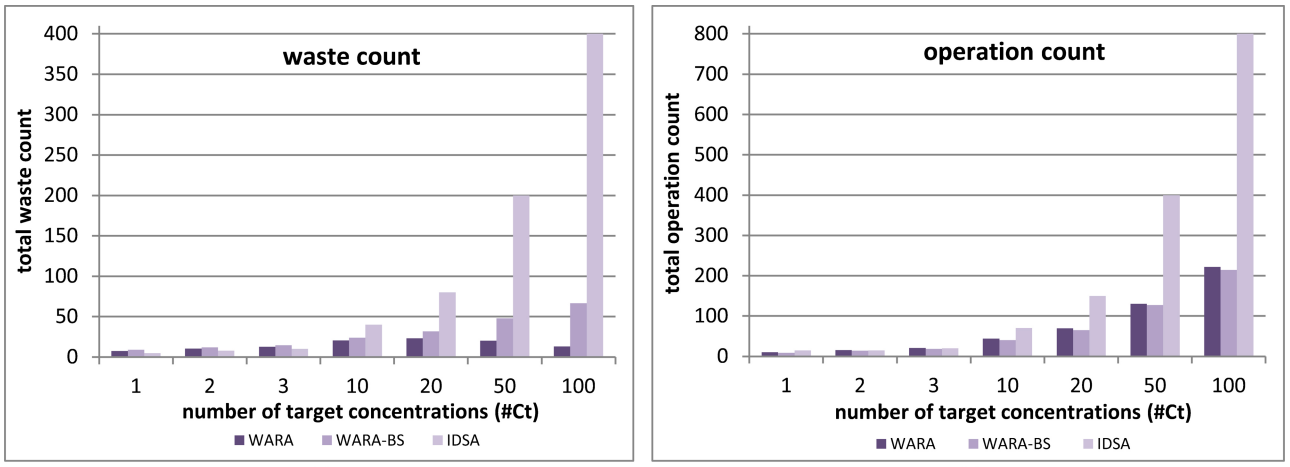


Fig. 15. Comparisons among WARA, WARA-BS, and IDSA.

Hence, an RCP with the largest gain should be selected first for droplet replacement to achieve a maximal possible reactant reduction.

In the previous definition, both nodes within an RCP must be reusable nodes. Consider the case illustrated in Fig. 10(a), no more feasible RCPs can be found for reactant minimization. However, if an additional PCV node is allowed while creating an RCP, it is likely that the overall ERU can be further minimized, just as Fig. 10(b) demonstrates. Consequently, the original definition of RCP is relaxed—for an RCP p, at most one of its paired nodes can be a PCV node as long as $gain(p)$ is positive. A loose upper bound on the number of maximum possible RCPs in a dilution graph G is C_2^k, where k is the number of all branch and PCV nodes in G.

If two or more RCPs are with the same largest gain, which is not uncommon, a secondary key for comparison is required for a better sorting outcome. As mentioned, if an RCP (x, y) is selected for droplet replacement, neither x nor y can appear in other RCPs later on since x and y are reused. Suppose that RCP $p = (w, x)$, RCP $q = (w, y)$, $\text{gain}(p) = \text{gain}(q)$, x merely appears in one RCP p, and y appears in several other RCPs besides q. Under such assumption, p should have higher precedence over q because of two reasons: 1) p is the only chance for x to be reused and 2) y still has other chances to be reused later even though the selection of p automatically invalidates q due to w.

To better model the above effect, the appearance count of node x, denoted as $ac(x)$, is defined. If x is a PCV node, then $ac(x)$ is set to the infinite; otherwise

$$ac(x) = |\{p | p \text{ is an RCP and } x \in p\}|. \tag{7}$$

Obviously, the smaller the value of $ac(x)$ is, the fewer chances (i.e., RCPs) x gets for being reused. Then, the uniqueness of RCP $p = (x, y)$, denoted as $uniq(p)$, is accordingly defined as

$$uniq(p) = min\{ac(x), ac(y)\}. \tag{8}$$

As previously explained, the smaller the value of $uniq(p)$ is, the higher precedence p has. Consequently, the uniqueness of RCP is used as the secondary key during RCP ordering.

Fig. 11 demonstrates an instance of droplet replacement utilizing two different types of RCPs. Fig. 11(a) gives an output right after droplet sharing. In the first run, the best RCP (i.e., the one with the highest precedence), which consists of two reusable nodes, is selected and the outcome is shown in Fig. 11(b). In the next run, the new best RCP, which consists of a reusable node and a newly introduced PCV node, is chosen and the result is reported in Fig. 11(c). The process of droplet replacement is not terminated until no RCP can be identified. Fig. 12 outlines the proposed droplet replacement flow.

Droplet sharing uses one existing waste droplet to replace an existing subtree, whereas droplet replacement needs to perform an extra interpolated dilution operation before replacing an existing subtree. As a result, if a subtree can be removed by either droplet sharing or droplet replacement, droplet sharing should be preferred. That is why WARA performs droplet sharing before droplet replacement.

VI. Experimental Results

To evaluate the proposed algorithm WARA, we compare it with an existing state-of-the-art method for multitarget preparation, IDSA [28]. To ensure the comparisons are appropriate, we adopt the same experimental environment setup reported in [28], where various numbers of target concentrations, ranging from 1 to 100, are considered, and every target concentration is randomly selected between 1/1024 and 1023/1024 (i.e., precision level = 10). To make the results more convincing, every reported value is an average of 1000 random cases in this paper instead of 20 in [28].

Fig. 13 illustrates the experimental flow and Table I shows the results right after the end of three consecutive optimization phases, respectively. Recall that the result of the first phase is also the result of REMIA [32]. Four counts for reactant (#R), buffer (#B), waste (#W), and operation (#OP), are reported accordingly. Note that these experimental data are collected after the unified optimal exponential dilution (OUED) process is performed. Each row gives the results for a specified number of target concentrations ($\#C_t$). Note

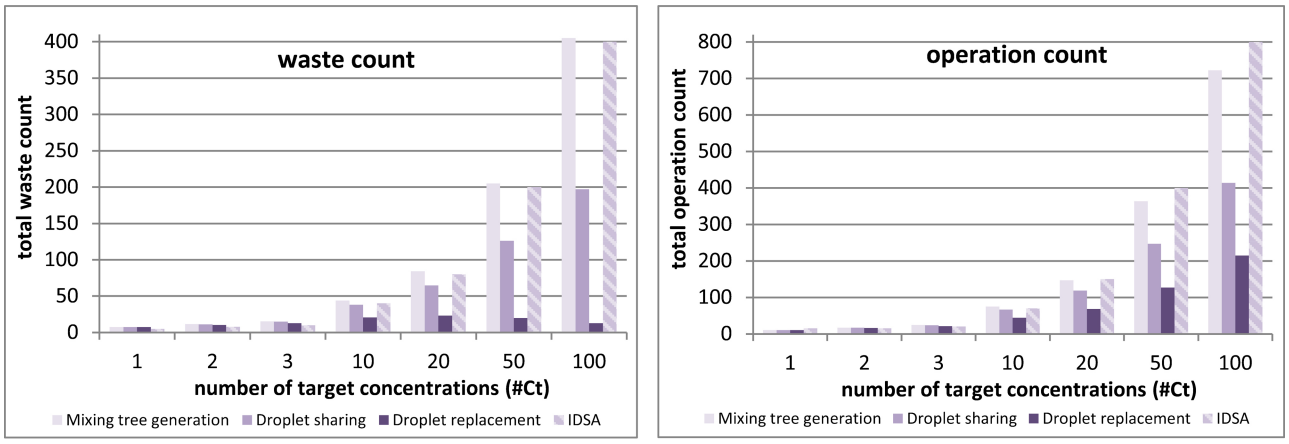


Fig. 16. Comparisons among the three phases of WARA and IDSA.

TABLE I
REACTANT/BUFFER/WASTE/OPERATION COUNTS AFTER THE THREE OPTIMIZATION PHASES OF WARA

	Mixing Tree Generation [32]				Droplet Sharing				Droplet Replacement			
$\#C_t$	#R	#B	#W	#OP	#R	#B	#W	#OP	#R	#B	#W	#OP
1	2.38	6.11	7.49	10.08	2.38	6.11	7.49	10.08	2.38	6.11	7.49	10.08
2	4.17	9.19	11.36	17.23	4.14	9.10	11.24	17.03	3.98	8.50	10.48	15.92
3	5.97	12.49	15.46	24.57	5.90	12.11	15.01	23.88	5.40	10.37	12.77	20.76
10	18.62	35.17	43.79	74.98	17.76	30.18	37.94	66.36	12.59	18.13	20.72	44.30
20	36.92	67.15	84.07	147.05	33.84	50.94	64.77	118.43	18.89	24.29	23.18	68.36
50	92.09	162.74	204.83	363.27	77.28	99.06	126.33	246.63	31.78	38.08	19.86	127.07
100	184.10	321.24	405.34	722.02	138.72	158.47	197.19	413.42	52.92	59.70	12.62	214.86

that the first row represents a special case in which only single target is considered. In this specific case, droplet sharing and replacement make no improvement at all. However, it is absolutely correct since it can find neither a pair of branch nodes x and y in an SMT with $\mathrm{CV}(x) = \mathrm{CV}(y)$ nor a feasible RCP. Hence, it is evident that REMIA performs very well in single-target sample preparation. Besides, the remaining rows (i.e., multitarget cases) indicate a trend that the improvement due to droplet sharing and droplet replacement becomes more significant as $\#C_t$ increases.

Fig. 14 basically presents the same set of data as in Table I but on a per-target basis to highlight this trend. It is evident that the benefit from the unified exponential dilution process is generally saturated roughly at $\#C_t = 20$; however, both droplet sharing and droplet replacement can constantly improve the outcome as $\#C_t$ gradually grows to 100. The difference between the final result and that of the first phase actually indicates the improvement from REMIA to WARA in multitarget sample preparation. Furthermore, droplet replacement contributes more than droplet sharing in both reactant and waste minimization. Overall, compared with REMIA, WARA reduces #R/#W/#OP by 10%/17%/16% when $\#C_t$ is merely three. As $\#C_t$ increases to 100, the reduction of reactant usage is 71%; the reduction of operation count is 70%, which should be considered notable since WARA does not pay extra attention on minimizing the operation count; and more significantly, the waste amount is reduced by 97%, which concludes that WARA is very effective in waste minimization.

Fig. 15 illustrates the results of three different multitarget sample preparation techniques, including WARA, WARA with BS (WARA-BS), and IDSA. WARA-BS is a variant of WARA that does not adopt REMIA but applies the BS method [25] to produce initial mixing trees in the first phase. The BS method guarantees the minimal number of dilution operations for single-target preparation. Also note that only the waste count and operation count were reported as the results of IDSA in [28]. Fig. 15 shows that all three approaches perform almost equally well in both waste and operation minimization when $\#C_t \leq 3$. As $\#C_t$ continues increasing, WARA significantly outperforms IDSA. Specifically, as $\#C_t = 10$, WARA produces 48% less waste and requires 37% fewer operations than IDSA; as $\#C_t = 100$, the reduction further becomes 97% and 73%, respectively. Fig. 15(a) reveals that WARA-BS produces more waste than WARA, which implies REMIA provides a better starting point than the BS method in waste reduction. Fig. 15(b) shows that WARA-BS performs slightly better than

WARA in terms of operation count, and the main reason is that BS requires fewer operations than REMIA in the first phase. Fig. 15 also demonstrates that WARA-BS outperforms IDSA as well, which suggests the contribution jointly from droplet sharing and droplet replacement is more significant than that from initial tree generation.

Fig. 16 presents the comparison results among the three phases of WARA and IDSA. It shows that the first phase (i.e., mixing tree generation) of WARA achieves almost the same quality of result as IDSA, which implies the combination of REMIA and the optimal unified exponential dilution technique is already a fairly good solution for multitarget preparation. Finally, WARA is very time-efficient as well—it can finish a case of $\#C_t = 100$ in just few seconds.

VII. Conclusion

Sample preparation is a fundamental process in biochemical reactions. Several techniques have been proposed to address this issue in past few years, while few of them focus on the multitarget sample preparation problem. In this paper, we proposed a new algorithm WARA aiming at both reactant and waste minimization in multitarget sample preparation. WARA first generates a set of the reactant-minimized mixing trees (i.e., SMTs) for each target concentration as its initial solution, and then successively apply droplet sharing and droplet replacement (i.e., waste droplet recycling) to further reduce the reactant usage and waste amount. The experimental results demonstrated that all three phases of WARA have their own contributions during optimization. The results also showed that WARA outperforms the existing state-of-the-art algorithm IDSA in terms of waste amount and operation count. Lastly, WARA is also very efficient in runtime. As a consequence, it is concluded that WARA is a better alternative for multitarget sample preparation on digital microfluidic biochips.

References

[1] A. Arora, G. Simone, G. B. Salieb-Beugelaar, J. T. Kim, and A. Manz, "Latest developments in micro total analysis systems," *Anal. Chem.*, vol. 82, no. 12, pp. 4830–4847, Jun. 2010.

[2] R. B. Fair, A. Khlystov, T. D. Tailor, V. Ivanov, R. D. Evans, P. B. Griffin, V. Srinivasan, V. K. Pamula, M. G. Pollack, and J. Zhou, "Chemical and biological applications of digital-microfluidic devices," *IEEE Design Test Comput.*, vol. 24, no. 1, pp. 10–24, Jan. 2007.

[3] M. G. Pollack, R. B. Fair, and A. D. Shenderov, "Electrowetting-based actuation of liquid droplets for microfluidic applications," *Appl. Phys. Lett.*, vol. 77, no. 11, pp. 1725–1726, Sep. 2000.

[4] P. Paik, V. K. Pamula, and R. B. Fair, "Rapid droplet mixers for digital microfluidic systems," *Lab Chip*, vol. 3, no. 4, pp. 253–259, Sep. 2003.

[5] V. Srinivasan, V. K. Pamula, and R. B. Fair, "An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids," *Lab Chip*, vol. 4, no. 4, pp. 310–315, May 2004.

[6] T.-Y. Ho, K. Chakrabarty, and P. Pop, "Digital microfluidic biochips: Recent research and emerging challenges," in *Proc. IEEE/ACM/IFIP Int. Conf. Hardware/Software Codesign Syst. Synthesis*, Oct. 2011, pp. 335–343.

[7] T.-Y. Ho, J. Zeng, and K. Chakrabarty, "Digital microfluidic biochips: A vision for functional diversity and more than Moore," in *Proc. IEEE/ACM Int. Conf. Computer-Aided Design*, Nov. 2010, pp. 578–585.

[8] M. Alistar, E. Maftei, P. Pop, and J. Madsen, "Synthesis of biochemical applications on digital microfluidic biochips with operation variability," in *Proc. Symp. Design, Test, Integr. Packaging MEMS/MOEMS*, May 2010, pp. 350–357.

[9] L. Luo and S. Akella, "Optimal scheduling of biochemical analyses on digital microfluidic systems," *IEEE Trans. Autom. Sci. Eng.*, vol. 8, no. 1, pp. 216–227, Jan. 2011.

[10] P.-H. Yuh, C.-L. Yang, and Y.-W. Chang, "Placement of defect-tolerant digital microfluidic biochips using the T-tree formulation," *ACM J. Emerging Technol. Comput. Syst.*, vol. 3, no. 3, pp. 13:1–13:32, Nov. 2007.

[11] Z. Xiao and E. F. Y. Young, "Placement and routing for cross-referencing digital microfluidic biochips," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 30, no. 7, pp. 1000–1010, Jul. 2011.

[12] M. Cho and D. Z. Pan, "A high-performance droplet routing algorithm for digital microfluidic biochips," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 27, no. 10, pp. 1714–1724, Oct. 2008.

[13] Y. Zhao and K. Chakrabarty, "Co-optimization of droplet routing and pin assignment in disposable digital microfluidic biochips," in *Proc. ACM Int. Symp. Phys. Design*, Mar. 2011, pp. 69–76.

[14] C. C.-Y. Lin and Y.-W. Chang, "Cross-contamination aware design methodology for pin-constrained digital microfluidic biochips," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 30, no. 6, pp. 817–828, Jun. 2011.

[15] T.-W. Huang, S.-Y. Yeh, and T.-Y. Ho, "A network-flow based pin-count aware routing algorithm for broadcast-addressing EWOD chips," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 30, no. 12, pp. 1786–1799, Dec. 2011.

[16] P. Roy, H. Rahaman, R. Bhattacharya, and P. Dasgupta, "A best path selection based parallel router for DMFBs," in *Proc. IEEE Int. Symp. Electron. Syst. Design*, Dec. 2011, pp. 176–181.

[17] D. Mitra, S. Ghoshal, H. Rahaman, K. Chakrabarty, and B. B. Bhattacharya, "On residue removal in digital microfluidic biochips," in *Proc. ACM Great Lakes Symp. VLSI*, May 2011, pp. 391–394.

[18] K. Chakrabarty, "Design automation and test solutions for digital microfluidic biochips," *IEEE Trans. Circuits Syst. I, Reg. Papers*, vol. 57, no. 1, pp. 4–17, Jan. 2010.

[19] S. Saha, A. Chakraborti, and S. Roy, "An efficient single-fault detection technique for micro-fluidic based biochips," in *Proc. IEEE Int. Conf. Adv. Comput. Eng.*, Jun. 2010, pp. 10–14.

[20] G. M. Walker, N. Monteiro-Riviere, J. Rouse, and A. T. O'Neill, "A linear dilution microfluidic device for cytotoxicity assays," *Lab Chip*, vol. 7, no. 2, pp. 226–232, Oct. 2007.

[21] H. Ren, V. Srinivasan, and R. B. Fair, "Design and testing of an interpolating mixing architecture for electrowetting-based droplet-on-chip chemical dilution," in *Proc. IEEE Int. Conf. Solid State Sensors, Actuators Microsyst. Transducers*, Jun. 2003, pp. 619–622.

[22] H. Moon, A. R. Wheeler, R. L. Garrell, J. A. Loo, and C. J. Kim, "An integrated digital microfluidic chip for multiplexed proteomic sample preparation and analysis by MALDI-MS," *Lab Chip*, vol. 6, no. 9, pp. 1213–1219, Jul. 2006.

[23] E. J. Griffith, S. Akella, and M. K. Goldberg, "Performance characterization of a reconfigurable planar-array digital microfluidic system," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 25, no. 2, pp. 345–357, Feb. 2006.

[24] T. Xu, V. K. Pamula, and K. Chakrabarty, "Automated, accurate, and inexpensive solution-preparation on a digital microfluidic biochip," in *Proc. IEEE Biomed. Circuits Syst. Conf.*, Nov. 2008, pp. 301–304.

[25] W. Thies, J. P. Urbanski, T. Thorsen, and S. Amarasinghe, "Abstraction layers for scalable microfluidic biocomputing," *Natural Comput.*, vol. 7, no. 2, pp. 255–275, May 2008.

[26] S. Roy, B. B. Bhattacharya, and K. Chakrabarty, "Optimization of dilution and mixing of biochemical samples using digital microfluidic biochips," *IEEE Trans. Computer-Aided Design Integr. Circuits Syst.*, vol. 29, no. 11, pp. 1696–1708, Nov. 2010.

[27] S. Roy, B. B. Bhattacharya, and K. Chakrabarty, "Waste-aware dilution and mixing of biochemical samples with digital microfluidic biochips," in *Proc. IEEE/ACM Design, Autom. Test Eur. Conf. Exhibition*, Mar. 2011, pp. 1059–1064.

[28] Y.-L. Hsieh, T.-Y. Ho, and K. Chakrabarty, "On-chip biochemical sample preparation using digital microfluidics," in *Proc. IEEE Biomed. Circuits Syst. Conf.*, Nov. 2011, pp. 297–300.

[29] S. Roy, B. B. Bhattacharya, P. P. Chakrabarti, and K. Chakrabarty, "Layout-aware solution preparation for biochemical analysis on a digital microfluidic biochip," in *Proc. IEEE Int. Conf. VLSI Design*, Jan. 2011, pp. 171–176.

[30] D. Mitra, S. Roy, K. Chakrabarty, and B. B. Bhattacharya, "On-chip sample preparation with multiple dilutions using digital microfluidics," in *Proc. IEEE Comput. Soc. Annu. Symp. VLSI*, Aug. 2012, pp. 314–319.

[31] S. Roy, P. P. Chakrabarti, and B. B. Bhattacharya, "Algorithms for on-chip solution preparation using digital microfluidic biochips," in *Proc. IEEE Comput. Soc. Annu. Symp. VLSI*, Aug. 2012, pp. 7–8.
[32] J.-D. Huang, C.-H. Liu, and T.-W. Chiang, "Reactant minimization during sample preparation on digital microfluidic biochips using skewed mixing trees," in *Proc. IEEE/ACM Int. Conf. Computer-Aided Design*, Nov. 2012, pp. 377–383.
[33] T.-W. Chiang, C.-H. Liu, and J.-D. Huang, "Graph-based optimal reactant minimization for sample preparation on digital microfluidic Biochips," in *Proc. IEEE Int. Symp. VLSI Design, Autom. Test*, Apr. 2013, pp. 79–82.
[34] D. A. Huffman, "A method for the construction of minimum redundancy codes," in *Proc. Inst. Radio Eng.*, Sep. 1952, pp. 1098–1101.

Juinn-Dar Huang (M'96) received the B.S. and Ph.D. degrees in electronics engineering from National Chiao Tung University, Hsinchu, Taiwan, in 1992 and 1998, respectively.

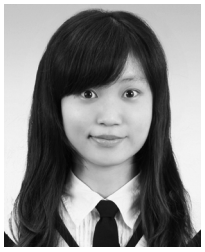
He is currently an Associate Professor in the Department of Electronics Engineering and the Institute of Electronics, National Chiao Tung University. He served in the Organizing Committees of IEEE/ACM ASP-DAC 2010. He was the Secretary General of Taiwan IC Design Society from 2004 to 2008, and the Technical Program Committee Vice-Chair of VLSI Design/CAD Symposium 2008. He served as a Technical Program Committee member of IEEE/ACM DATE in 2008 and 2010, ASP-DAC from 2012 to 2013, and IEEE VLSI-DAT from 2010 to 2013.

Dr. Huang is a member of the ACM, IEICE, and Phi Tau Phi.

Chia-Hung Liu (SM'08) received the B.S. degree in electronics engineering from National Chiao Tung University, Hsinchu, Taiwan, in 2007, where he is currently pursuing the Ph.D. degree in the Department of Electronics Engineering.

His current research interests include high-level synthesis, VLSI design automation, and computer-aided design for digital microfluidic biochips.

Mr. Liu has been the chair of the IEEE NCTU student branch from 2010 to 2011.



Huei-Shan Lin received the B.S. degree in electrical engineering from National Sun Yat-Sen University, Kaohsiung, Taiwan, in 2011, and the M.S. degree in electronics engineering from National Chiao Tung University, Hsinchu, Taiwan, in 2013.

Her current research interests include VLSI design automation and computer-aided design for digital microfluidic biochips.