Waste-Aware Single-Target Dilution of a Biochemical Fluid using Digital Microfluidic Biochips

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Abstract

A key challenge in design automation of digital microfluidic biochips is to carry out on-chip dilution/mixing of biochemical samples/reagents for achieving a desired concentration factor (CF). In a bioassay, reducing the waste is crucial because the management of waste droplets is cumbersome and expensive; transporting them to the waste reservoir and washing their pathways to clean contaminated regions and remove the residue left by them may increase assay completion time. The existing dilution algorithms attempt to reduce the number of mix-split steps required in the process but focus little on minimization of sample requirement or waste droplets. In this work, we characterize the underlying combinatorial properties of waste generation and identify the inherent limitations of two earlier mixing algorithms *twoWayMix* [Thies et al., *Natural Computing*, 2008] and *DMRW* [Roy et al., *TCAD*, 2010] in addressing this issue. Based on these properties, we design an improved dilution/mixing algorithm (*IDMA*) that optimizes the usage of intermediate droplets generated during the dilution process, which in turn, reduces the demand of sample/reagent and production of waste. The algorithm terminates in $O(d)$ steps for producing a target CF with a maximum error of $\frac{1}{2^{d+1}}$, where d is the an accuracy level of the desired CF . Based on simulation results for all CF values ranging from $\frac{1}{1024}$ to $\frac{1023}{1024}$ using a sample (100% concentration) and a buffer solution (0% concentration), we present an integrated scheme of choosing the best waste-aware dilution algorithm among four approaches such as *twoWayMix*, *DMRW*, *REMA* [Huang et al., *ICCAD*, 2012] and *IDMA*, for a single target CF . Finally, an architectural layout of a DMF biochip that supports the proposed scheme is designed.

Keywords: Biochips, computer-aided-design, digital microfluidics, dilution, mixing, waste minimization.

1. Introduction

The recent emergence of digital microfluidic (DMF) biochips has led to a paradigm shift in many healthcare-related application areas, e.g., point-of-care clinical diagnostics, high-throughput sequencing, toxicity monitoring and proteomics [2–6]. In DMF biochips, electrical actuation is normally used to manipulate (e.g., transport, merge, split, mix, dispense) discrete droplets of nanoliter or picoliter volume of sample/reagent flu-

ids on a two-dimensional array of electrodes [5, 7]. A schematic diagram of the cross-sectional view of a basic cell of a DMF biochip is shown in Fig. 1 [2]. Detailed descriptions and architectures of such biochips are available in the literature [2].

An efficient design of a microfluidic biochip mandates a complete integration of multiple assay operations such as detection, sample pre-treatment, sample mixing, and dilution control on one single chip [8]. The dilution problem arises in numerous biochemical assays for the preparation of samples or mixtures, e.g., cDNA for real-time PCR, immunoassays for detecting cy-

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Figure 1: (a) Top view of a digital microfluidic biochip, and (b) Cross-sectional view of a cell at a detection site.

tokines in serum samples, which is a marker for inflammation. A bioassay may require several values of concentration (or dilution) factor of a sample or a reagent during its execution on a DMF chip. One example of a real assay is enzymatic glucose assay (Trinder’s reaction); it uses a dilution factor of 200 or more [8]. Another example is the automated DMF-based protocol used for the extraction of proteins from heterogeneous fluids by precipitation [9]. This method requires several reagents with different concentration levels such as 50 mg/mL of BSA solution (sample), 20% TCA (precipitant), 70/30 v/v chloroform/acetonitrile (rinse solution) and 100 mM borate buffer containing 1% SDS (resolubilizing buffer).

A key challenge in designing DMF biochips and mapping lab-bench protocols to it is to automate the dilution process of a biochemical sample such that a desired concentration factor is achieved while minimizing the number of mix-split steps and the requirement of input fluids. In order to reduce the consumption of expensive sample and reagent fluids, the number of waste droplets should thus be minimized during an automated sample preparation procedure. Note that the future generation of biochips is being scaled down in size with the availability of nanoliter-sized on-chip fluid reservoirs, and they are capable of handling picoliter-volume of discrete fluid droplets [10]. As such chips can be used for multiplexing several protocols on-chip, the minimization of waste droplets produced during the execution of a bioassay is even more crucial.

Note that larger the amount of waste-droplet production, higher will be the overhead for transporting them to the waste reservoir, and for washing their pathways to clean contaminated

regions and remove the residue therein. This may significantly impact assay completion time. The time needed for waste-droplet transportation, may however, be reduced by increasing the number of on-chip waste reservoirs, but again, this is not a good option. Hence, the reduction of waste droplets is desirable for lessening various on-chip droplet-transportation overheads and cost. Unfortunately, it is not yet well understood what kind of sample preparation algorithm will lead to minimum waste production.

Main Results: In this paper, we identify and analyze the inherent limitations of three existing dilution algorithms namely, *twoWayMix* [11], *DMRW* [12], *REMIA* [13], from the perspective of waste generation during the automation of single-target dilution procedure. We characterize the underlying combinatorial properties of discrete dilution process and waste generation. Based on these properties, we design a new dilution algorithm called Improved Dilution/Mixing Algorithm (*IDMA*) that maximizes the reuse of intermediate droplets generated during the process.

The proposed algorithm terminates in $O(d)$ steps for producing a target concentration factor (CF) with a maximum error in CF of $\frac{1}{2^{d+1}}$, where d is the accuracy level of CF and is user-defined. Given a target CF , we also propose an Integrated Dilution Scheme (*IDS*) for selecting the most efficient scheme from the perspective of waste reduction among *twoWayMix*, *DMRW*, *REMIA* and *IDMA*. In order to implement the integrated dilution scheme *IDS*, we design an architectural layout of electrodes for DMF-based biochips consisting of a $O(d)$ -size rotary mixer, one merge-and-split type (1 : 1) mixer module, and a constant number of storage electrodes. The proposed methodology will help users in selecting the best solution for the automation of on-chip sample pre-processing.

The organization of the remainder of the paper is as follows. The related prior work on dilution algorithms and the motivating examples are discussed in Section 2. Section 3 discusses the proposed dilution algorithm along with related theoretical results. An integrated dilution scheme for single-target sample-preparation of two reactant fluids is presented in Sec-

tion 4. An architectural layout of electrodes for implementing the proposed algorithm is described in Section 5. Simulation results of the integrated dilution scheme are presented in Section 6. Finally, the paper is concluded in Section 7.

2. Automated Dilution of Fluids: Prior Work and Motivation

2.1. Prior Work

DMF biochips typically deal with discrete droplets on a uniform 2D-array of equi-sized electrodes, hence their volumes are usually an integral multiple of that of a single droplet (unit volume). There are various $(k : \ell)$ mixing models that are often used, where k -unit volume of one substance is mixed with ℓ -unit volume of another substance to produce $(k + \ell)$ -unit volume of the resultant mixture in a single mixing operation. Three such cases are: (i) $k = \ell = 1$, (ii) $k = \ell \neq 1$, and (iii) $k \neq \ell$, where k, ℓ are positive integers. The first case, i.e., $(1 : 1)$ mixing model is easy to implement and hence it is widely used in practice.

The *concentration factor* (CF) is defined as the ratio of the initial volume of the sample to the final volume of the diluted sample. Dilution is a special case of mixing two substances, where one of them is a buffer (neutral) solution with $CF = 0$. If the samples of CF C_1 and C_2 are mixed in a volumetric ratio of $k : \ell$, then the resulting sample will have CF $C_r = \frac{k.C_1 + \ell.C_2}{k + \ell}$, and a volume of $(k + \ell)$ units, assuming that the same buffer is used in diluting both the samples.

As mentioned earlier, in a DMF biochip, biochemical samples can only be mixed using discrete volumes of liquid droplets. Therefore, it is possible to carry out dilution in only discrete steps to approximate the desired concentration level. In $(1 : 1)$ -mixing model, two unit-volume droplets are mixed to obtain a new CF , and the resulting two-unit-volume droplet is split into two unit-volume droplets, out of which one is used in the next step of dilution and the other may not be used further (this is called a waste droplet). Thus, it is a challenge to achieve a desired CF using the fewest mix-split steps. The dilution algorithm reported by Griffith et al. [14] outputs a sequence of $(1 : 1)$ mix-split steps to be performed in order to

achieve a certain target CF . Thies et al. [11] reported a dilution/mixing algorithm for minimizing the total number of mix-split steps. Subsequently, many other algorithms with various objectives appeared in the literature for automated sample preparation [11, 15–18]. In general, the goal in these algorithms is to minimize the number of mix-split steps (i.e., mixing time), reactant usage, storage requirement, or waste production. In some approaches, the same cost (per unit volume) is assigned to both sample and buffer fluids [1, 12, 19, 20]. These algorithms are primarily focused on the minimization of waste droplets, which indeed minimizes the total cost of input fluids (sample and buffer). In another important approach to sample preparation, different costs are assigned to various input reactant fluids. Based on this variable-cost metric, several algorithms for minimization of reactant usage were reported [13, 21–24].

The state-of-the-art dilution algorithms can be broadly classified into two categories: (a) single-target dilution algorithms and (b) multi-target dilution algorithms (in which droplets of different concentrations need to be prepared at the same time). Most of the earlier work on single-target dilution aim to minimize the number of mix-split steps, wastage or reactant usage. These challenges become more critical for the multi-target dilution problem because an optimal sharing of intermediate droplets (some of which would have been otherwise wasted), is needed for the reduction of time and cost of the overall procedure. During the last few years, several heuristic approaches were proposed in the literature for both single and multi-target sample preparation problems [13, 15, 19–22, 24–26].

Hsieh et al. proposed two algorithms namely, *IDSA* [21] and *RSM* [22], for preparing multiple targets to achieve waste minimization. Mitra et al. [15] reported a dilution algorithm based on de Bruijn graphs for generating droplets with multiple CF s without using any intermediate storage. Huang et al. [13] introduced an algorithm called *REMIA* with an objective of minimizing reactant usage as well as wastage for solving the multi-target dilution problem. Bhattacharjee et al. [19, 25] proposed a pruning-based dilution algorithm to reduce wastage during the generation of diluted sample with multiple concen-

trations.

Another multi-target sample preparation algorithm is proposed by Huang et al. [26], which is referred to as *WARA*. It extensively exploits the ideas of waste recycling and intermediate droplet sharing to reduce both reactant usage and wastage. Note that the algorithm *WARA* reduces to *REMI*A [13] for the single-target dilution problem. Liu et al. [24] presented an algorithm namely *Ext-CoDOS* for multi-target dilution problem, which identifies the possibilities of sharing two common dilution operations for reactant minimization. Dinh et al. [20] considered the objective of reducing reactant usage and waste production in one model. They proposed an algorithm based on a network-flow model, which can be tuned to optimize the desired objective. It is capable of optimizing the cost of reactants and loss of waste droplets even in a multi-target sample preparation scenario.

Another important practical issue was stated as an open problem by Thies et al. [11]: if two inputs are related (e.g., a sample of 10% acid and a sample of 20% of the same acid), then how to produce a desired dilution of the sample (say, 17%). Most of the existing algorithms consider the problem of automated dilution of a fluid from the supply of two pure (100%) input fluids and they do not discuss generalized dilution from multiple arbitrary concentrations. Some of the recently published work considered this issue and provided a solution [1, 12, 14, 29]. Table 1 indicates the scope of the proposed work against earlier approaches, where the entry “Yes/No” indicates the applicability of the concerned method (T_{ms} is the total num-

ber of (1 : 1) mix-split steps required to produce target droplets).

2.2. Motivation of the Work

In this paper, we focus only on the single-target dilution problem and assume that both sample and buffer solution have the same cost per unit volume. Hence, in this case, waste minimization will automatically minimize the total cost of reactants used. We consider *twoWayMix* [11], *DMRW* [12], and *REMI*A [13] as three representative algorithms for waste minimization, and use them in our comparative study.

Note that after one (1 : 1) mix operation, the *CF* of the mixture becomes the mean value of those of the two source droplets. Thus, for the sake of convenience, the *CF* of a target dilution (C_t , $0 \leq C_t < 1$), is expressed as a d -bit binary fraction, when an accuracy level of d is desired. In other words, the *CF* of a target droplet can be expressed as $\frac{x}{2^d}$, where $x \in \mathbb{Z}^+$, $0 \leq x \leq (2^d - 1)$. Also, a buffer solution can be assumed to have *CF* = 0 ($= \frac{0}{2^d}$), and a pure sample can be assumed to have *CF* = 1 ($= \frac{2^d}{2^d}$). For our study, we assume that $d = 10$, and a sample (100% conc.) and a buffer (0% conc.) are given; hence, a target *CF* value that lies between $\frac{1}{1024}$ to $\frac{1023}{1024}$ will be of interest to us.

We first analyze the performance of two dilution algorithms namely, *twoWayMix* [11] and *DMRW* [12], for several *CF*s over the range from $\frac{1}{1024}$ to $\frac{1023}{1024}$. The *twoWayMix* always uses either sample or buffer solution at each (1 : 1) mix-split step to mix with the current droplet. Thus, it consumes only one droplet at every mix-split step to continue the process, and produces one waste droplet at each step (excepting the last one, where the target dilution is achieved). For example, the mix-split procedure for the target *CF* $C_t = \frac{341}{1024}$ for the *twoWayMix* is illustrated in Fig. 2, where the number of waste droplets is nine.

Note that this sequence of mix-split steps can be conveniently represented by a directed graph as shown in Fig. 2 [12]. Each node of this graph represents a *CF* value; the top and bottom nodes represent the sample ($CF = 1 = \frac{1024}{1024}$) and the buffer ($CF = 0 = \frac{0}{1024}$) respectively. These two nodes have in-degree zero. The node with out-degree zero (in this case $\frac{341}{1024}$) rep-

Table 1: Scope and results of the proposed work compared to prior art.

Dilution Algorithm [†]	# Target <i>CF</i> s (# Droplets)	Minimization Criteria			Arbitrary Input <i>CF</i> s
		T_{ms}	Wastage	Reactant Usage	
Griffith et al. [14]	Single (2)	No	No	No	Yes
<i>twoWayMix</i> [11]	Single (2)	Yes	No	No	No
<i>DMRW</i> [12]	Single (2)	No	Yes	No	Yes
Roy et al. [1]	Single (2)	No	Yes	No	Yes
<i>MTDG</i> [16–18, 27]	Single (≥ 2)	Yes	No	No	No
[13, 21–24]	One or More (2)	No	No	Yes	No
<i>DBG</i> A [15, 28]	Multiple (2)	Yes	No	No	No
<i>PBDA</i> [19]	Multiple (2)	No	Yes	No	No
<i>WARA</i> [26]	Multiple (2)	No	Yes	Yes	No
<i>NFSP</i> [20]	One or More (≥ 2)	No	Yes	Yes	No
<i>GDA</i> , <i>EGDA</i> [29, 30]	Single (2)	Yes	No	No	Yes
Proposed Scheme	Single (2)	Yes	Yes	No	Yes

represents the target CF . Other nodes have in-degree two, and out-degree one, each. If a node v_i has two predecessor nodes v_j and v_k , one of them (say, v_j) must be either the sample node or the buffer node according to the requirement of *twoWayMix* algorithm; the other predecessor node (v_k) indicates a droplet of $CF = v_k$ produced at the last dilution step, and a (1 : 1) mixture of v_j and v_k produces two droplets of $CF = v_i$. For example, the node $(\frac{640}{1024})$ has two predecessor nodes $(\frac{256}{1024})$ and $(\frac{1024}{1024})$; the two directed edge towards the node $(\frac{640}{1024})$ represents the fact that one droplet of $CF = \frac{256}{1024}$ is mixed with one droplet of $CF = \frac{1024}{1024}$, and then the mixture is split to produce two droplets of intermediate $CF = \frac{640}{1024}$. As only one of the resulting droplets is needed in the next step, one droplet is wasted at each step but the last one (denoted by a black dot adjacent to a node).

In the *twoWayMix* algorithm, each droplet of an intermediate CF value is required only once in the mix-split sequence, whereas in the *DMRW* algorithm, it may be needed multiple times in order to reach a target CF . Thus, to meet the demand of intermediate CF values, these droplets are to be produced starting from the given supply of sample and buffer fluids. As a result, *DMRW* may need a larger number of (1 : 1) mix-split steps compared to *twoWayMix*; however, the number of waste droplets is reduced significantly. In order to implement *DMRW*, a layout architecture with a rotary mixer was used for performing ($k : k$) mixing ($k \geq 1$, where $k \in \mathbb{Z}^+$) in one step [12].

In the *DMRW* method, it is assumed that a sample with two initial CF s, C_ℓ and C_h , is supplied such that and the desired target CF C_t satisfies $0\% \leq C_\ell \leq C_t < C_h \leq 100\%$. Initially, C_ℓ and C_h are considered as the lower- and upper-boundary CF s respectively. At every mix-split step, it always mixes only two

Figure 2: Mix-split steps obtained by *twoWayMix* for the target CF $C_t = \frac{341}{1024}$ ($\equiv 0.01010101_2$).

boundary CF s. After such a (1 : 1) mixing, it compares the resulting CF (C_i) with C_t . If they are equal within the error margin of $\frac{1}{2^{d+1}}$, the target CF is said to have reached; otherwise, if C_i is less (greater) than C_t , then we replace the lower-boundary (upper-boundary) CF by C_i . This process is repeated until the target CF C_t is reached. Note that at every mix/split step, the interval (i.e., the range of CF s) between the lower and upper boundaries not only includes the target CF , but also continues to shrink; it finally converges to the target CF satisfying the desired error margin within at most d steps.

As before, the complete mix-split sequence $Z_m, m \leq d$, that leads to the target CF can be conveniently depicted using a weighted digraph $\mathcal{M}(V, E)$, where a node $v \in V$ denotes a CF value. The edge set E is defined as follows: when a 1X-volume droplet of $CF = C_i$ is mixed with another 1X-volume of droplet of $CF = C_j$ to produce a mixture of CF C_k (i.e., $C_k = \frac{C_i + C_j}{2}$), we represent this fact by putting two directed edges in the graph (v_i, v_k) and (v_j, v_k) .

For example, consider the mix-split digraph for the target $CF = \frac{127}{1024}$ shown in Fig. 3(a). Here, the initial boundary CF s, which are supplied, are $\frac{0}{1024}$ and $\frac{1024}{1024}$. Note that for the sake of clarity of the diagram, the nodes in the digraph are not shown as explicit bubbles; they are implicitly assumed to be present at the two ends of every edge. In this digraph, the initial boundary nodes have in-degree zero and every other node has in-degree exactly two. Only the target node has out-degree zero; the out-degree of other nodes may lie between 1 to d . In *DMRW*, the digraph $\mathcal{M}$ representing the mix-split sequence Z_m for a target CF is usually drawn in a special manner to reflect gradual shrinkage of the bounding interval with respect to the target; for example, in Fig. 3(a), we mix $\frac{0}{1024}$ and $\frac{1024}{1024}$ to produce $\frac{512}{1024}$; this is shown by the two directed edges incident on it from them. Imagine a virtual vertical line that passes through the target node. Since the intermediate CF ($\frac{512}{1024}$) is higher than the target CF , the node $\frac{512}{1024}$ is drawn on the right side of the virtual line. In the next step, $\frac{256}{1024}$ is produced by mixing the two current boundary CF s ($\frac{0}{1024}$ and $\frac{512}{1024}$), and this process is continued until the target is reached. A directed edge incident on a node i from the

Figure 3: Digraphs obtained by *DMRW* [12] for the target CF (a) $C_t = \frac{127}{1024}$, (b) $C_t = \frac{513}{1024}$, (c) $C_t = \frac{16}{1024}$, and (d) $C_t = \frac{341}{1024}$, using the supply of droplets with CF s $C_l = \frac{0}{1024}$ and $C_h = \frac{1024}{1024}$.

(left) right predecessor is called a (left-) right-edge of i .

The sequence of intermediate CF values that leads to the target from the two initial boundary values is given by the longest directed path (i.e., of length m) to the target in $\mathcal{M}$. Also, from a visual perspective, the CF -values corresponding to the nodes of the graph $\mathcal{M}$ ordered from C_l to C_h via C_t , appear in an ascending order when the graph is embedded on a plane. For example, in Fig. 3(b) this order is $\frac{0}{1024}, \frac{256}{1024}, \frac{320}{1024}, \dots, \frac{341}{1024}, \frac{342}{1024}, \frac{344}{1024}, \dots, \frac{1024}{1024}$. For some other example target CF s $C_t = \frac{513}{1024}$, $C_t = \frac{16}{1024}$ and $C_t = \frac{341}{1024}$, the corresponding mix-split sequences (or digraphs), the number of sample, buffer, and waste droplets are shown in Fig. 3(b)-3(d), respectively.

Note that with each directed edge (v_i, v_j) of $\mathcal{M}$, an integer weight $D(i)$ is attached; this is indicative of how many 1X volume droplets, with CF value denoted by the node v_i , are to be produced to complete the dilution process. These weights can be found by retracing the graph from the target node C_t ,

where two 1X droplets will be produced at the end [12]. In this context, we define the skew of a node as follows. The skew $Sk(i)$ of a node i in a digraph is its weighted out-degree, i.e., the sum of the weights of all the outgoing-edges from node i . In case of $C_t = \frac{127}{1024}$, the node with $CF = \frac{128}{1024}$ is demanded multiple times (sum of the outgoing edge-weights is 7) as presented in Fig. 3(a). Hence, for this node i with $CF = \frac{128}{1024}$, the skew $Sk(i) = 7$. Similarly, for $C_t = \frac{513}{1024}$, $Sk(\frac{512}{1024}) = 9$ and for $C_t = \frac{16}{1024}$, $Sk(\frac{0}{1024}) = 6$, as shown in Figs. 3(b) and 3(c), respectively. However, for $C_t = \frac{341}{1024}$ as depicted in Fig. 3(d), the skew value of all the nodes is less than or equal to two. It may be observed here that only one droplet is wasted (which is the minimum) in contrast to nine when the *twoWayMix* method is run (Fig. 2). Also, it is easy to note that the weight of a directed edge (v_i, v_j) is equal to $D(i) = \lceil \frac{Sk(i)}{2} \rceil$, if we assume a $(k : k)$ mixing model.

A close look at Figs. 3(a)-3(d) reveals that the skew of a node representing an intermediate CF in the digraph has a strong impact on waste droplet generation as well as on the total number of $(1 : 1)$ mix-split steps. This follows from the fact that in each $(1 : 1)$ mix-split step, two droplets of an intermediate CF are produced and therefore, the demand of this node as well as wastage is optimized, when both of these two droplets are consumed in the subsequent mix-split steps. The above observation motivates us to further reduce the number of waste droplets generated in *DMRW* [12], by controlling the skew of an intermediate CF s node.

3. Proposed Dilution Algorithm: *IDMA*

We now present a new heuristic-based dilution algorithm, called Improved Dilution/Mixing Algorithm (*IDMA*) for waste minimization based on the *DMRW* approach. Assume that we are supplied with two input fluids, one with lower CF C_l and another with higher CF C_h . The objective is to produce a target droplet of CF C_t with an accuracy level of d , where $0 \leq C_l \leq C_t < C_h \leq 1$. First, *DMRW* is run to construct the digraph $\mathcal{M}$ corresponding to the sequence Z_m of m $(k : k)$ mix-split steps, $m \leq d$. The execution of *IDMA* needs the following

preprocessing steps.

1. Approximate C_ℓ as $\frac{c}{2^d}$ for the accuracy level d , where c, d are positive integers with $0 \leq c < 2^d$ and $d \geq 1$.
2. Represent C_ℓ as $\frac{a}{2^d}$ and C_h as $\frac{b}{2^d}$, where a, b are positive integers with $0 \leq a < b \leq 2^d$.

Table 2 lists the notation used in this paper. Note that the workload of one $(k : k)$ mix-split step can be assumed to be that of $k(1 : 1)$ mix-split steps. Thus, T_{ms} for a $(k : k)$ mix-split step is computed as accordingly.

For any digraph, we do the following steps to compute the performance parameters.

1. Set the skew of $node(C_\ell)$ as two, i.e., $Sk(node(C_\ell)) = 2$.
2. Backtrace the digraph in reverse topological order starting from node $node(C_\ell)$. For each node v_i , consider the left-edge (v_ℓ, v_i) and the right-edge (v_r, v_i) incident on v_i ; set their weights $In_\ell(i) = In_r(i) = \left\lfloor \frac{Sk(i)}{2} \right\rfloor$.
3. For each edge (v_i, v_j) , compute the edge-weight $D(i)$, i.e., the number of droplets of $CF(i)$ to be produced, as $D(i) = In_\ell(i) + In_r(i) = 2 \left\lfloor \frac{Sk(i)}{2} \right\rfloor$, whereas the number of droplets of $CF(i)$ used subsequently is $Sk(i)$. Thus, the number of waste droplets with $CF(i)$ can be obtained as $W(i) = D(i) - Sk(i)$. Note that for any node i , $W(i)$ is only 1 or 0.

Table 2: Notation used in this paper.

Symbols	Meaning
Z_m	Sequence of m mix-split steps ($m \leq d$) obtained by <i>DMRW</i>
$\mathcal{M}$	Digraph or dilution tree representing Z_m
$Z_{m'}$	Modified sequence of m' mix-split steps obtained by <i>IDMA</i>
$\mathcal{M}'$	Modified digraph representing $Z_{m'}$ obtained by <i>IDMA</i>
$N(C_i)$	Node in a digraph corresponding $CF = C_i$
$CF(i)$	CF corresponding to a node i in the digraph
$CF_{left}(i)$	CF of the left predecessor of the node i in a digraph
$CF_{right}(i)$	CF of the right predecessor of the node i in a digraph
$Sk(i)$	Skew of a node i in a digraph
Sk_{max}	Maximum skew of any node in a digraph
$D(i)$	The integer weight associated with a directed edge (v_i, v_j) , i.e., the number of droplets to be produced whose CF is denoted by the node v_i in a mix-split digraph
$In_r(i) (I_\ell(i))$	Weight attached to the right(left)-edge incident on node i
$O(i)$	Out-degree of a node i in a digraph
$U(i)$	Number of demanded droplets of the CF denoted by node i in a digraph
T_{ms}	Total number of $(1 : 1)$ mix-split steps in a digraph
W	Total number of waste droplets generated
$W(i)$	Number of waste droplets with the CF denoted by the node i in a digraph
WL_ℓ	Workload on the linear mixer capable of $(1 : 1)$ mixing
WL_r	Workload on the rotary mixer capable of $(k : k)$ mixing

4. The total number of waste droplets generated is $W = \sum_i W(i)$.
5. Scan the digraph to compute the total number of $(1 : 1)$ mix-split steps as $T_{ms} = \sum_i In_\ell(i)$.

3.1. Algorithm Overview: *ImproveDigraph*

The proposed dilution algorithm *IDMA* uses a heuristic-based procedure *ImproveDigraph*, which first modifies the digraph $\mathcal{M}$ obtained by *DMRW* [12]. Let $\mathcal{M}'$ be the modified digraph corresponding to the modified sequence $Z_{m'}$ of m' $(k : k)$ mix-split steps obtained by *ImproveDigraph*, where $m' \geq m$. Note that in *DMRW*, if the intermediate CF values appear alternately on the left (right) and right (left) side with respect to the target CF as in Fig. 3(d), the skew of these nodes will be exactly two. In these cases, both the droplets generated at a node are consumed without any wastage. Thus, when $Sk(i)$ of a node i is greater than two, we attempt to find a modified bounding interval by changing the lower-boundary CF (or the upper-boundary CF) depending on the target CF value. In order to achieve this, a few mix-split steps are executed starting from the current boundary values to produce the new boundary CF . We write a procedure *SkewModify(skNode)* that performs this task to modify a digraph, in which the node $skNode$ is the first node in the mix-split sequence graph obtained by *DMRW* whose skew $Sk > 3$. This simple heuristic applied on the digraph obtained from *DMRW* can significantly reduce both W and T_{ms} . The procedure *ImproveDigraph* is described as **Algorithm 1**.

Algorithm *ImproveDigraph* preserves the same accuracy level of d in the target CF as in *DMRW*. Since the execution of *DMRW* requires $O(d)$ time, the overall time complexity of *ImproveDigraph* is also $O(d)$. For an illustration of *ImproveDigraph*, consider the digraphs of Fig. 3(a) and Fig. 3(b) obtained by the *DMRW* method for two target CF s $C_\ell = \frac{127}{1024}$ and $C_\ell = \frac{513}{1024}$; when **Algorithm 1** is run, we get two modified digraphs as shown in Fig. 4(a) and 4(b), respectively. Note that in Fig. 4(a), we generate a modified upper-boundary CF ($\frac{192}{1024}$), and in Fig. 4(b), we modify the lower-boundary CF to ($\frac{384}{1024}$).

Algorithm 1: *ImproveDigraph*($C_\ell, C_h, C_t, m, \mathcal{M}$)

- 1: Obtain the sequence Z_m of $(k : k)$ mix-split steps (nodes) from the digraph $\mathcal{M}$.
 - 2: **for** each node i in Z_m **do**
 - 3: Compute the skew of node i in $\mathcal{M}$ as $Sk(i)$.
 - 4: **end for**
 - 5: **if** there is no node in Z_m with $Sk(i) \geq 3$ **then**
 - 6: $\mathcal{M}' = \mathcal{M}$.
 - 7: **Exit**.
 - 8: **else**
 - 9: $skNode = \text{First node with } Sk(skNode) \geq 3 \text{ in the sequence } Z_m$.
 - 10: $\mathcal{M}' = \text{Subgraph of } \mathcal{M} \text{ corresponding to the sequence up to node } skNode$.
 - 11: **end if**
 - 12: Execute *SkewModify*($skNode$).
 - 13: Execute *DMRW*(L, R, C_t, m) and obtain a digraph $\mathcal{M}''$.
 - 14: Obtain the modified digraph $\mathcal{M}' = \mathcal{M}' \cup \mathcal{M}''$.
 - 15: Update the sequence $Z_{m'}$, where $m' \geq m$, corresponding to $\mathcal{M}'$.
-

Algorithm 2: *SkewModify*($skNode$)

- 1: **if** $CF(skNode) = \frac{1}{2}$ **then**
 - 2: **if** $CF(skNode) < C_t$ **then**
 - 3: $L = CFleft(skNode)$ and $R = \lceil CF(skNode) + CFright(skNode) \rceil / 2$.
 - 4: Add $node(R)$ and two directed edges $\{CF(skNode), R\}$ and $\{CFright(skNode), R\}$ to $\mathcal{M}'$.
 - 5: **else**
 - 6: $L = \lceil CF(skNode) + CFleft(skNode) \rceil / 2$ and $R = CFright(skNode)$.
 - 7: Add $node(L)$ and two directed edges $\{CF(skNode), L\}$ and $\{CFleft(skNode), L\}$ to $\mathcal{M}'$.
 - 8: **end if**
 - 9: **else**
 - 10: **if** $CF(skNode) < C_t$ **then**
 - 11: $L = \lceil CF(skNode) + CFleft(skNode) \rceil / 2$ and $R = CFright(skNode)$.
 - 12: Add $node(L)$ and two directed edges $\{CF(skNode), L\}$ and $\{CFleft(skNode), L\}$ to $\mathcal{M}'$.
 - 13: **else**
 - 14: $L = CFleft(skNode)$ and $R = \lceil CF(skNode) + CFright(skNode) \rceil / 2$.
 - 15: Add $node(R)$ and two directed edges $\{CF(skNode), R\}$ and $\{CFright(skNode), R\}$ to $\mathcal{M}'$.
 - 16: **end if**
 - 17: **end if**
-

Figure 4: Digraphs obtained by *ImproveDigraph* for (a) $C_t = \frac{127}{1024}$ and (b) $C_t = \frac{513}{1024}$ of W and T_{ms} .

3.2. Theoretical Analysis

Consider the target $CF = \frac{341}{1024}$, for which ten $(1 : 1)$ mix-split steps are required by *DMRW* as shown in Fig. 3(d). Note that out of ten mix-split steps executed in this case, a droplet is wasted only in one step. This observation motivates us to analyze the combinatorial properties of a mix-split sequence, which are responsible for the production of waste droplets. This analysis also leads to a powerful heuristic called “one-third rule”, which is used in **Algorithm 1**.

Note that in the mix-split digraph obtained by *DMRW*, if the progression of the sequence towards the target CF alternates between the left (right) and right (left) side of the target, then the out-degree of each node can never exceed two, and therefore, the number of waste droplets is optimized and no additional demand of intermediate CF s is created. Our analysis reveals

that such an alternating mix-split sequence is possible, resulting in two target droplets of $CF = C_t$ with only one waste droplet. For example, if $d = 10$, $C_\ell = \frac{0}{2^d}$ and $C_h = \frac{2^d}{2^d}$, this happens for $C_t = \frac{341}{1024}$ or $C_t = \frac{683}{1024}$. These two target CF s correspond to the case where a sample is mixed with a buffer in a ratio of (1 : 2), or (2 : 1). In other words, the number 341 (683) appears nearly at the one-third (two-third) position of the interval between 0 to 1024 (see Fig. 5).

It may be observed that, if the target CF is at one-third position of the interval $(C_h - C_\ell)$, i.e., $\frac{b-a}{2^d}$, where $C_h = \frac{b}{2^d}$ and $C_\ell = \frac{a}{2^d}$, then during the next mix-split step (in the sequence of alternate steps) the input fluid droplet with lower CF (C_ℓ) is mixed with the resultant droplet of the previous step to get a new intermediate CF (**Case-I**). On the other hand, if the target CF is at two-third position of the interval $(C_h - C_\ell)$, then during the next mix-split step the input fluid droplet with higher CF (C_h) is mixed with the resultant droplet of the previous step to get the new intermediate CF (**Case-II**). An alternating mix-split steps are then executed until the target CF is reached. The result can be generalized in the form of the following theorem.

Figure 5: Scale of the input CF s $C_\ell = \frac{a}{2^d}$ and $C_h = \frac{b}{2^d}$.

Theorem 1. Starting from two boundary CF s $C_\ell = \frac{a}{2^d}$ and $C_h = \frac{b}{2^d}$, where $0 \leq C_\ell < C_h \leq 1$, an alternating sequence of i (1 : 1) mix-split steps will lead to two droplets of target CF $C_i = \frac{c_i}{2^d}$ with only one waste droplet, where:

$$c_i = \frac{1}{3} \left[\left(2a + \frac{(-1)^{i+1}}{2^{i+1}} a \right) + \left(b + \frac{(-1)^i}{2^{i+1}} b \right) \right] \text{ (Case-I)} \quad (1)$$

or

$$c_i = \frac{1}{3} \left[\left(a + \frac{(-1)^i}{2^{i+1}} a \right) + \left(2b + \frac{(-1)^{i+1}}{2^{i+1}} b \right) \right] \text{ (Case-II)} \quad (2)$$

where i is a non-negative integer ranging from 0 to $(\lfloor \log_2(b-a) \rfloor - 1)$.

Proof. **Theorem 1** can be proved by solving a recurrence relation as follows. The recurrence relation on the consecutive

resultant CF s obtained by the *DMRW* method can be written as

$$\begin{aligned} c_i &= \frac{1}{2}(c_{i-1} + c_{i-2}) \\ \therefore 2c_i - c_{i-1} - c_{i-2} &= 0 \end{aligned} \quad (3)$$

We consider **Case-I** first, i.e., C_ℓ is mixed with C_0 to obtain C_1 . Thus, the initial conditions are: $c_0 = \frac{a+b}{2}$ and $c_1 = \frac{1}{2}(\frac{a+b}{2} + a) = \frac{3a+b}{4}$.

Next, we solve this recurrence to prove that Eqn. (1) is true. Let $c_i = r^i$ be a ("guess") solution of the recurrence relation in Eqn. (3) for some undetermined value of r . Then, we have the equation

$$2r^2 - r - 1 = 0$$

as the characteristic equation for the homogeneous recurrence relation in Eqn. (3) and the two distinct roots of this equation are the characteristic roots given by $r_1 = 1$ and $r_2 = -\frac{1}{2}$. So, the general solution to the recurrence relation can be written as

$$\begin{aligned} c_i &= \alpha_1(r_1)^i + \alpha_2(r_2)^i \\ \therefore c_i &= \alpha_1(1)^i + \alpha_2\left(-\frac{1}{2}\right)^i \end{aligned} \quad (4)$$

where α_1 and α_2 are real constants.

From Eqn. (4), we can determine the values of c_0 and c_1 . To find α_1 and α_2 we plug-in the initial conditions to Eqn. (4):

$$\begin{aligned} \alpha_1 + \alpha_2 &= \frac{a+b}{2} \quad (\text{putting } i = 0) \\ \text{and } \alpha_1 + \alpha_2 \cdot \left(-\frac{1}{2}\right) &= \frac{3a+b}{4} \quad (\text{putting } i = 1) \end{aligned}$$

Solving these equations we get the values as $\alpha_1 = \frac{2a+b}{3}$ and $\alpha_2 = \frac{-(a-b)}{6}$. Thus, we get an exact formula for c_i as follows:

$$\begin{aligned} c_i &= \alpha_1 + \alpha_2 \cdot \frac{(-1)^i}{2^i} \\ &= \frac{2a+b}{3} - \left(\frac{a-b}{6}\right) \cdot \frac{(-1)^i}{2^i} \\ &= \frac{1}{3} \left[(2a+b) + \frac{(-1)^{i+1}}{2^{i+1}} \cdot (a-b) \right] \\ &= \frac{1}{3} \left[\left(2a + \frac{(-1)^{i+1}}{2^{i+1}} a \right) + \left(b + \frac{(-1)^i}{2^{i+1}} b \right) \right] \end{aligned}$$

Thus, by solving the recurrence relation on c_i obtained by *DMRW*, Eqn. (1) is proved. For **Case-II**, i.e., C_h is mixed with C_0 to produce C_1 , we obtain $c_1 = \frac{1}{2}(\frac{a+b}{2} + b) = \frac{a+3b}{4}$ as ini-

tial conditions; by solving the recurrence relation of Eqn. (3) Eqn. (2) can then be proved similarly. This completes the proof. $\square$

For *IDMA*, it is not straightforward to obtain a relation between the initial *CF*s (C_ℓ and C_h) and the number of waste droplets (W). Note that the number of sequential mix-split steps (m') required by *IDMA* to achieve the target *CF* C_t starting from two initial boundary *CF*s $C_\ell = \frac{a}{2^d}$ and $C_h = \frac{b}{2^d}$ of a fluid, where $0 \leq C_\ell \leq C_t < C_h \leq 1$, is $(\lfloor \log_2(b-a) \rfloor - 1)$. Again, the total number of waste droplets generated is $W = \sum_{i=1}^{m'} W(i)$. Also, for any mix-split step, i , $W(i)$ is either 1 or 0 as a $(k:k)$ mix-split model is assumed.

If $C_\ell = \frac{0}{2^d}$ (buffer solution with 0% conc.) and $C_h = \frac{2^d}{2^d}$ (sample fluid with 100% conc.), then Eqn. (1) and Eqn. (2) are reduced to:

$$c_i = \frac{2^d}{3} \left[\left(1 + \frac{(-1)^i}{2^{i+1}} \right) \right] \quad (5)$$

and

$$c_i = \frac{2^d}{3} \left[\left(2 + \frac{(-1)^{i+1}}{2^{i+1}} \right) \right] \quad (6)$$

respectively, where where i is a non-negative integer between 0 and $(\lfloor \log_2 2^d \rfloor - 1)$, i.e., $(d-1)$.

Fig. 3(d) depicts the digraph for target *CF* $\frac{341}{1024}$ indicating 10 (1:1) mix-split steps and the intermediate *CF*s as $C_0 = \frac{512}{1024}$, $C_1 = \frac{256}{1024}$, $C_2 = \frac{384}{1024}$, $C_3 = \frac{320}{1024}$, $C_4 = \frac{352}{1024}$, $C_5 = \frac{336}{1024}$, $C_6 = \frac{344}{1024}$, $C_7 = \frac{340}{1024}$, $C_8 = \frac{342}{1024}$ and $C_9 = \frac{341}{1024}$. These *CF* values can be obtained from Eqn. (5) by setting $d = 10$ and $i = 0, 1, \dots, 9$. In order to generate all these target *CF*s, only one droplet will be wasted. Similar target *CF*s for which $W = 1$, can be found from Eqn. (6) by putting $d = 10$ and $i = 0, 1, \dots, 9$. The result stated in **Theorem 1** can now be used to reduce the production of waste droplets (W) in the dilution process by modifying the mix-split sequence of *DMRW*. When the skew of a node in the mix-split graph of *DMRW* is greater than two, we attempt to generate two intermediate *CF*s C'_ℓ and C'_h , from the two input *CF*s C_ℓ and C_h , so that the target *CF* C_t is (nearly) equal to one-third (i.e., $\frac{1}{3}$ for **Case-I**) or two-third (i.e., $\frac{2}{3}$ for **Case-II**) of the interval $(C'_h - C'_\ell)$. We refer to this rule as the *One-Third Rule*,

and use it in the proposed scheme *IDMA* (**Algorithm 3**) for the reduction of wastage in the dilution process.

3.3. Algorithm Overview: IDMA

In the proposed scheme *IDMA*, first *ImproveDigraph* is invoked, and then the *One-Third Rule* is applied iteratively to further reduce the total number of waste droplets (W) generated in *DMRW* [12], by controlling the skew of an intermediate node. However, we have observed that it may not be easy to determine a suitable interval of two *CF*s such that the target *CF* is (nearly) equal to one-third or two-third of the interval. Hence, we mimic the one-third rule in a slightly different way while implementing *IDMA*.

For any target *CF* C_t with a desired accuracy level of d and the supply of two input *CF*s C_ℓ and C_h , *IDMA* first constructs the modified digraph M' by calling *ImproveDigraph* (**Algorithm 1**). If in M' , if there is any node whose skew value is more than two, or appears later compared to that in the mix-split sequence of the previous graph M , two new boundary *CF*s are again determined based on the one-third rule. *IDMA* iterates

Algorithm 3: *IDMA*($C_\ell, C_h, C_t, m, \mathcal{M}$)

- 1: Execute *ImproveDigraph*($C_\ell, C_h, C_t, m, \mathcal{M}$) and obtain the digraph $\mathcal{M}'$.
 - 2: Obtain the sequence $Z_{m'}$ of $(k:k)$ mix-split steps (nodes) from the digraph $\mathcal{M}'$, where $m' \geq m$.
 - 3: **for** each node i in $Z_{m'}$ **do**
 - 4: Compute the skew of node i in $\mathcal{M}'$ as $Sk(i)$.
 - 5: **end for**
 - 6: **if** there is no node i in $Z_{m'}$ with $Sk(i) \geq 3$ **then**
 - 7: Obtain the modified digraph as $\mathcal{M}'$.
 - 8: **Exit**.
 - 9: **else**
 - 10: $skNode = \text{First node with } Sk(skNode) \geq 3 \text{ in the sequence } Z_{m'}$.
 - 11: $\mathcal{M}' = \text{Subgraph of } \mathcal{M}' \text{ corresponding to the sequence up to node } skNode$.
 - 12: **end if**
 - 13: **while** $skNode \neq \text{NULL}$ **do**
 - 14: Execute *SkewModify*($skNode$).
 - 15: Execute *DMRW*(L, R, C_t, m) and obtain a digraph $\mathcal{M}''$.
 - 16: Obtain the modified digraph $\mathcal{M}' = \mathcal{M}' \cup \mathcal{M}''$.
 - 17: Update the sequence $Z_{m'}$, where $m' \geq m$, corresponding to $\mathcal{M}'$.
 - 18: **if** there is no node i that appears later in the sequence than the $skNode$ in $Z_{m'}$ with $Sk(i) \geq 3$ **then**
 - 19: Obtain the modified digraph as $\mathcal{M}'$.
 - 20: Set $skNode = \text{NULL}$.
 - 21: **else**
 - 22: $skNode = \text{First node with } Sk(skNode) \geq 3 \text{ in the sequence } Z_{m'} \text{ later in the sequence than the previous } skNode$.
 - 23: $\mathcal{M}' = \text{Subgraph of } \mathcal{M}' \text{ corresponding to the sequence up to node } skNode$.
 - 24: **end if**
 - 25: **end while**
-

Figure 6: Flowchart of the proposed method *IDMA*.

this process to modify the digraph $\mathcal{M}'$ obtained in the previous iteration, and whenever it finds a node in the digraph with $Sk() \geq 3$, the procedure *SkewModify()* is called to modify the digraph. A flowchart of *IDMA* is shown in Fig. 6. The pseudo-code of the proposed scheme *IDMA* is written as **Algorithm 3**.

3.4. Results of *IDMA*

For a target *CF*, such as $\frac{1}{1024}$ or $\frac{1023}{1024}$, some nodes in the mix-split digraph as obtained by *ImproveDigraph*, still have a high skew value. When *IDMA* is applied on the digraphs obtained by *DMRW* for two target *CF*s $C_t = \frac{327}{1024}$ and $C_t = \frac{702}{1024}$, we obtain two modified digraphs as shown in Fig. 7(a) and Fig. 7(b), respectively. Note that for both these targets, *IDMA* further reduces W as compared to *DMRW* or *ImproveDigraph*. For the target *CF* $\frac{327}{1024}$, the number of (1 : 1) mix-split steps (T_{ms}) is also reduced from that of *DMRW*, whereas for the target *CF* $\frac{702}{1024}$, T_{ms} is reduced compared to *ImproveDigraph*.

We use several parameters to compare performances of four different dilution schemes, namely *twoWayMix* [11], *DMRW* [12], *REMA* [13] and *IDMA*. The total number of sam-

Figure 7: Digraphs obtained by *IDMA* for (a) $C_t = \frac{327}{1024}$ and (b) $C_t = \frac{702}{1024}$.

Table 3: Comparative results of four methods for two target *CF*s $\frac{327}{1024}$ and $\frac{702}{1024}$.

Target <i>CF</i> , C_t	Method	W	T_{ms}	S	B	Sk_{max}
$\frac{327}{1024}$	<i>twoWayMix</i> [11]	9	10	5	6	1
	<i>DMRW</i> [12]	7	18	3	6	4
	<i>REMA</i> [13]	7	17	3	6	3
	<i>IDMA</i>	5	17	2	5	3
$\frac{702}{1024}$	<i>twoWayMix</i> [11]	8	9	7	3	1
	<i>DMRW</i> [12]	7	16	6	3	5
	<i>REMA</i> [13]	7	12	3	6	5
	<i>IDMA</i>	5	17	5	2	4

[†]In order to produce two target droplets, W : total number of waste droplets generated, T_{ms} : total number of (1 : 1) mix-split steps, S : total number of droplets of sample fluid, B : total number of droplets of buffer solution, Sk_{max} : the maximum skew value of any node in the digraph.

ple (buffer) droplets required to produce one/two target droplets is denoted as S (B). The maximum skew value of a node in a mix-split digraph is represented as Sk_{max} . Table 3 reports these parameters for two examples.

Figure 8: The decision tree followed in the integrated dilution scheme *IDS*.

4. Integrated Scheme for Single-Target Dilution of a Fluid

In this section, given target CF C_t , we present an Integrated Dilution Scheme (*IDS*) that selects the best dilution algorithm among *twoWayMix* [11], *DMRW* [12], *REMIA* [13], and *IDMA* from the perspective of waste minimization. Let the total number of waste droplets (W) for the digraph obtained by *IDMA*, *DMRW* [12], *twoWayMix* [11] and *REMIA* [13] be W_0 , W_1 , W_2 and W_3 , respectively. Furthermore, the total number of (1 : 1) mix-split steps (T_{ms}) in the sequence obtained by *IDMA*, *DMRW* [12], *twoWayMix* [11] and *REMIA* [13] be T_0 , T_1 , T_2 and T_3 , respectively. The following two theorems are immediate from the results stated in [11, 12].

Theorem 2. For every target CF , the number of waste droplets generated by *DMRW* is less than or equal to that produced by *twoWayMix*.

Theorem 3. For every target CF , the number of mix-split steps needed by *DMRW* is greater than or equal to that needed by *twoWayMix*.

From **Theorem 2** and **Theorem 3**, we observe that $W_1 \leq W_2$ and $T_1 \geq T_2$. Using a similar argument we can show that $T_0 \geq T_2$. The integrated scheme *IDS* aims to minimize waste (W) as the primary objective and the total number of (1 : 1) mix-split steps (T_{ms}) as the second objective. A decision tree shown in

Figure 10: Layout of electrodes for the integrated scheme *IDS*.

Fig. 8, depicts the scheme. The complete procedure is shown as a flowchart in Fig. 9.

5. Proposed Layout for On-chip Dilution

In order to expedite the dilution process by using *DMRW* or *IDMA*, an on-chip $(k : k)$ -mixer is needed, $k \leq 5$, over the range of 1023 discrete target CF s between $\frac{1}{1024}$ and $\frac{1023}{1024}$ [12]. It is observed that, when *IDMA* is chosen for 476 target CF s, k is at most 3 and *DMRW* is chosen for 526 target CF s, where k is at most 4; and for the remaining 21 cases where *twoWayMix* is chosen, where k is at most 1. This can be implemented using a rotary mixer [12, 31]. Our simulation experiments with *IDS* for $d = 10$ reveal that among 1023 target CF s, *IDMA* is chosen for 476 target CF s, where k is at most 3, and *DMRW* is chosen for 526 target CF s, where k is at most 4; for the remaining 21 cases *twoWayMix* is chosen, where $k = 1$. Hence, for single-target dilution of a fluid, an architectural layout of electrodes with a rotary mixer as shown in Fig. 10 can be used to implement *IDMA* or *DMRW*. A portion of the same layout can be used as a three-electrode array (linear) mixer [32] to perform a homogeneous (1 : 1) mixing as well. droplet [32].

5.1. Overheads of Linear and Rotary Mixers

For providing a relative comparison of the algorithms, we normalize the workload of a rotary mixer with respect to a linear mixer. For a given target CF , the workload on the rotary

Figure 9: Flowchart of the integrated dilution scheme *IDS*.

mixer (WL_r) is counted as $WL_r = \sum_i k$, where i varies over all $(k : k)$ mix-split steps with $k > 1$ used in the sequence, i.e., how many times the rotary mixing is needed for $k > 1$. The workload (WL_ℓ) on a linear mixer is counted as the number of $(1 : 1)$ mix-split-step is needed in the sequence. For several target *CF*s, we report the mixer-workloads needed by different dilution schemes.

5.2. Storage Unit Requirements

Once an on-chip $(k : k)$ mix-split step ($k \geq 1$) is executed by a linear or a rotary mixer, one or more droplets of the intermediate *CF* so produced may need to be temporarily stored on some electrodes for subsequent use. However, the storage requirement depends on the target *CF* as well the on the choice of dilution algorithm. Note that after a $(k : k)$ mix-split step, the volume of the mixture becomes $2k$ units. In the next mix-

split step, if only one $1X$ droplet is used, we will need at most $(2k - 1)$ storage cells to store the rest of it.

In *DMRW* or *IDMA*, only the two current boundary *CF*s are needed at any instant of time, and their most recent values are obtained in the last two steps of the concerned mix-split sequence. Hence, in our simulation experiment, we observe the maximum storage requirement (q) in two consecutive mix-split steps over all target *CF* values. We have observed that the value of q needed by *twoWayMix*, *DMRW* or *IDMA* with accuracy level $d = 10$ is 6. Based on this observation, we have designed our layout with on-chip storage electrodes (shown in ‘pink’ color) as in Fig. 10.

For two target *CF*s $\frac{327}{1024}$ and $\frac{702}{1024}$, the values of WL_r , WL_ℓ and q obtained by *twoWayMix* [11], *DMRW* [12], *REMIA* [13] and *IDMA* are shown in Table 4.

Table 4: Comparative results of four methods for two target CF s $\frac{327}{1024}$ and $\frac{702}{1024}$.

Target CF, C_t	Method	W	T_{ms}	S	B	Sk_{max}	WL_r	WL_ℓ	q
$\frac{327}{1024}$	<i>twoWayMix</i> [11]	9	10	5	6	1	0	10	0
	<i>DMRW</i> [12]	7	18	3	6	4	13	5	5
	<i>REMIA</i> [13]	7	17	3	6	3	2	9	4
	<i>IDMA</i>	5	17	2	5	3	12	5	4
$\frac{702}{1024}$	<i>twoWayMix</i> [11]	8	9	7	3	1	0	9	0
	<i>DMRW</i> [12]	7	16	6	3	5	11	5	5
	<i>REMIA</i> [13]	7	12	3	6	5	5	7	5
	<i>IDMA</i>	5	17	5	2	4	8	9	3

[†]In order to produce two target droplets, W : total number of waste droplets generated, T_{ms} : total number of (1 : 1) mix-split steps, S : total number of droplets of sample fluid, B : total number of droplets of buffer solution, Sk_{max} : the maximum skew value of any node in the digraph, WL_r : the workload on the rotary mixer, WL_ℓ : the workload on the linear mixer, q : total number of on-chip storage units required.

Theorem 4. *At any instant of time during the execution of DMRW or IDMA, intermediate-droplets of at most two distinct CF values need to be stored.*

Proof. Easily follows from the fact that only the two most-recent boundary values are needed, at any instant of time, during the execution of DMRW or IDMA. $\square$

Based on this result, we allocate the storage electrodes in an arrangement of two non-overlapping pipelines (on the two opposite sides of the output of the rotary mixer), as shown in Fig. 10.

6. Simulation Results

The simulation results of the proposed dilution algorithm IDMA, and the integrated dilution scheme IDS are reported in this section for an accuracy level of the desired CF as $d = 10$. It is observed that the maximum skew value Sk_{max} in a digraph can be reduced by IDMA over DMRW. For a comparative study, we have computed the following performance parameters — the number of waste droplets (W), the number of (1 : 1) mix-split steps (T_{ms}), the maximum skew value of a node (Sk_{max}) in the digraph, workload of the linear (rotary) mixer WL_ℓ (WL_r), and maximum on-chip storage requirement (q).

6.1. Test Data Set of Target CFs

As mentioned earlier, we have conducted our simulation experiment with IDS using all four dilution algorithms, namely *twoWayMix* [11], *DMRW* [12], *REMIA* [13] and *IDMA*, assuming $d = 10$, i.e., all 1023 CF values are studied between $\frac{1}{1024}$

and $\frac{1023}{1024}$. A buffer (with $CF = 0$) and the pure sample (with $CF = 1$) are assumed to be supplied as the two initial boundary fluids. The simulation results have been summarized in the following subsections.

6.2. Performance of IDS

Our results indicate that out of 1023 target CF s, IDMA is most suitable for waste minimization and for reducing T_{ms} for 435 CF s. Among the remaining CF s DMRW is found to be the best for 507 cases, whereas, REMIA performs best for other 61 CF s in terms of waste minimization. In the case of the remaining 20 target CF s, *twoWayMix* can be used for waste minimization, as it requires the minimum number of (1 : 1) mix-split steps, and is simple to implement. The pie-chart shown in Fig. 11 reflects this distribution of CF s into four methods, *twoWayMix*, *DMRW*, *REMIA* and *IDMA*. In order to illustrate the choice made by IDS, we consider a range of target CF s from $\frac{251}{1024}$ to $\frac{275}{1024}$ in Fig. 12.

For some target CF s, the comparative results of four dilution methods are shown in Table 5. In the case of target CF s $\frac{127}{1024}$, $\frac{251}{1024}$, $\frac{259}{1024}$, $\frac{263}{1024}$, $\frac{269}{1024}$, $\frac{313}{1024}$, $\frac{327}{1024}$, $\frac{513}{1024}$ and $\frac{702}{1024}$, IDMA provides the best reduction in wastage and T_{ms} compared to that by *twoWayMix*, DMRW or REMIA. Similarly, DMRW is the best suitable method for the target CF s, $\frac{261}{1024}$, $\frac{267}{1024}$ and $\frac{341}{1024}$ in waste minimization. For the target CF s $\frac{16}{1024}$ and $\frac{256}{1024}$, the best possible reduction in wastage with minimum T_{ms} is obtained by *twoWayMix*. Whereas, REMIA is the best choice for the target CF s $\frac{264}{1024}$ and $\frac{268}{1024}$.

As IDMA performs best for 435 CF s out of 1023 target CF s compared to *twoWayMix*, DMRW and REMIA, we report the comparative results of two important performance parameters (W and T_{ms}) in Fig. 13(a) and 13(b), respectively, only for

Figure 11: Distribution of 1023 target CF s for waste minimization by four methods *twoWayMix* [11] (20 CF s), *DMRW* [12] (507 CF s), *REMIA* [13] (61 CF s) and *IDMA* (435 CF s).

Figure 12: Selection of the best method among four methods *twoWayMix* [11], *DMRW* [12], *REMIA* [13] and *IDMA*, for different target *CFs* in the range of ($\frac{251}{1024}$ to $\frac{275}{1024}$) for waste minimization.

Figure 13: Histograms of four methods *twoWayMix* [11], *DMRW* [12], *REMIA* [13] and *IDMA* for 435 *CFs* out of 1023 target *CFs*. Distribution of (a) *W* values and (b) *T_{ms}* values, with the count of target *CFs*.

Table 5: Selection of the best method among *twoWayMix*, *DMRW*, *REMIA* and *IDMA* for waste minimization in case of some target *CFs*.

Target	<i>IDMA</i>		<i>DMRW</i> [12]		<i>twoWayMix</i> [11]		<i>REMIA</i> [13]		Best Scheme
<i>CF, C_t</i>	<i>W₀</i>	<i>T₀</i>	<i>W₁</i>	<i>T₁</i>	<i>W₂</i>	<i>T₂</i>	<i>W₃</i>	<i>T₃</i>	
$\frac{16}{1024}$	5	6	5	6	5	6	5	6	<i>twoWayMix</i>
$\frac{127}{1024}$	3	11	7	14	9	10	7	14	<i>IDMA</i>
$\frac{251}{1024}$	5	17	6	13	9	10	7	13	<i>IDMA</i>
$\frac{256}{1024}$	1	2	1	2	1	2	1	2	<i>twoWayMix</i>
$\frac{259}{1024}$	5	17	8	15	9	10	7	10	<i>IDMA</i>
$\frac{261}{1024}$	5	17	5	13	9	10	7	10	<i>DMRW</i>
$\frac{263}{1024}$	5	15	8	16	9	10	7	11	<i>IDMA</i>
$\frac{264}{1024}$	5	16	5	10	6	7	5	7	<i>REMIA</i>
$\frac{267}{1024}$	5	16	5	13	9	10	6	10	<i>DMRW</i>
$\frac{268}{1024}$	5	16	5	11	7	8	5	8	<i>REMIA</i>
$\frac{269}{1024}$	5	13	5	14	9	10	7	11	<i>IDMA</i>
$\frac{313}{1024}$	5	15	7	18	9	10	6	11	<i>IDMA</i>
$\frac{327}{1024}$	5	17	7	18	9	10	6	11	<i>IDMA</i>
$\frac{341}{1024}$	1	10	1	10	9	10	5	10	<i>DMRW</i>
$\frac{513}{1024}$	2	10	9	14	9	10	9	10	<i>IDMA</i>
$\frac{702}{1024}$	5	17	7	16	8	9	7	12	<i>IDMA</i>

these 435 *CFs*. Fig. 13(a) reveals that *IDMA* can significantly reduce the total number of waste droplets (*W*) in producing a single-target dilution of a fluid compared to that by *twoWayMix*,

DMRW and *REMIA*. Moreover, Fig. 13(b) indicates that for several target *CFs*, *IDMA* can further reduce the total number of (1 : 1) mix-split steps (*T_{ms}*) required to produce a single-target dilution in comparison with that by *DMRW*, while *IDMA* may require little more *T_{ms}* compared to *REMIA*. Similar to *DMRW*, *IDMA* can trade-off wastage and the number of (1 : 1) mix-split steps.

6.3. Performance Improvement by *IDMA* over *DMRW*

Fig. 14(a) depicts that for several target *CFs*, *IDMA* can further reduce *Sk_{max}* values in the modified digraphs compared to that obtained by *DMRW* [12]. For 435 *CFs*, the maximum of *Sk_{max}* values in a digraph obtained by *DMRW* can be 9, whereas *IDMA* can reduce this value to 5. However, in the case of *twoWayMix*, *Sk_{max}* of a digraph is always one. Fig. 14(b) and 14(c) show that the mix-split sequence obtained by *IDMA* consists of more (1 : 1) operations compared to that needed in *DMRW*, and hence, the former reduces the workload on the rotary mixer. Hence, for many target *CFs*, *IDMA* requires fewer number of storage units (*q*) in comparison to *DMRW*, as evident from the simulation result shown in Fig. 14(d).

Figure 14: Histograms of three methods *twoWayMix* [11], *DMRW* [12] and *IDMA* for 435 *CFs* out of 1023 target *CFs*. Distribution of (a) Sk_{max} values, (b) WL_ℓ values, (c) WL_r values, and (d) q values, with the count of target *CFs*.

7. Conclusions

In this paper, we have presented an improved algorithm called *IDMA* for reducing the total number of waste droplets while diluting a reactant fluid with a buffer fluid on a DMF biochip. We have also proposed an integrated dilution scheme *IDS* that can be used to select the best option among four state-of-the-art dilution algorithms for waste minimization. We have designed an architectural layout of electrodes that can support this scheme for single-target dilution of a fluid. Simulation results support our findings. Several open problems stem out from this study. One of them is to design a dilution algorithm using the $(k : \ell)$ mixing model, where $k \neq \ell$ and $k, \ell \in \mathbb{Z}^+$. For example, in order to achieve a target $CF \frac{341}{1024}$ of a reactant fluid from the supply of the pure reactant and buffer fluids, ten $(1 : 1)$ mix-split steps are required by *twoWayMix*, *DMRW*, or *IDMA*. However, just one $(2 : 1)$ mix-split step is sufficient to produce

the target droplets (if two droplets of the buffer fluid and one droplet of the reactant fluid are combined). We leave these issues for possible future work.

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