Waste-Aware Dilution and Mixing of Biochemical Samples with 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 waste droplet handling is cumbersome and the number of waste reservoirs on-chip needs to be minimized to use limited volume of sample and expensive reagents and hence to reduce the cost of a biochip. 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 (BS algorithm by Thies et al., Natural Computing 2008; DMRW algorithm by Roy et al., IEEE 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 $O(n)$ steps for producing a target CF with a precision of $\frac{1}{2^n}$. 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 BS, DMRW, and IDMA for any given value of CF. Finally, an architectural layout of a DMF biochip that supports the proposed scheme is designed.**

I. 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 [1], [2], [3], [4], [5].

For an efficient design of a microfluidic biochip one has to integrate multiple assay operations such as detection, sample pre-treatment, sample mixing, and dilution control on one single chip [6]. The dilution problem arises in numerous biochemical assays for preparation of samples or mixtures, e.g., cDNA for real-time PCR, immunoassays for detecting cytokines in serum samples, which is a marker for inflammation. Several bioassays to be implemented on DMF biochips, may require any value of concentration (or the dilution factor) of the samples or reagents. One example of a real assay is enzymatic glucose assay (Trinder's reaction); it uses a dilution factor of 200 or more [6]. Another automated DMF-based protocol for extracting proteins from heterogeneous fluids by precipitation has been explained in [7]. 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).

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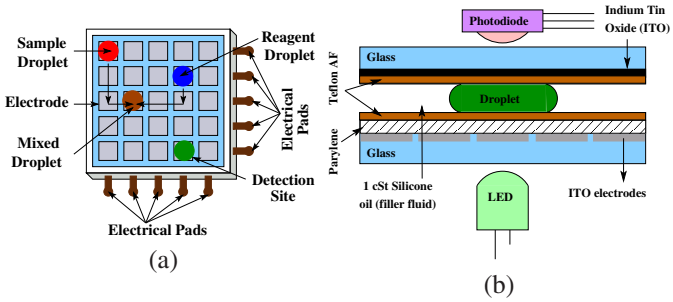

Fig. 1. (a) Top view of a digital microfluidic biochip, and (b) Cross-sectional view of a cell at detection site [1].

In many digital microfluidic (DMF) biochips, electrical actuation is used to manipulate (transporting, merging, splitting, mixing, dispensing, etc.) discrete droplets of nanoliter or picoliter volume of the sample/reagent fluids on a two-dimensional electrode array based on a special phenomenon called electrowetting-on-dielectric (EWOD) [8], [4]. A schematic diagram of the cross-sectional view of a basic cell of a DMF biochip is shown in Fig. 1. Descriptions and architectures of such biochips are available in the literature [1].

A key challenge in designing DMF biochips and mapping lab-bench protocols into it is to automatically carry out the dilution of a biochemical sample at a desired concentration factor within a small number of mix/split steps and with minimum requirement of sample fluids. In a bioassay reducing the waste is crucial as today's biochips are being scaled down in size and are capable of handling picoliter volume of discrete fluid droplets stored in nanoliter volume of on-chip fluid reservoirs. Hence, the number of waste droplet generation should be minimized to use limited volume of sample and expensive reagents. Furthermore, the waste droplet handling is cumbersome and the number of waste reservoirs on-chip needs to be minimized to reduce cost of a biochip. Reduction of sample and waste droplets also lessens the on-chip routing load for transportation. However, the dynamics of waste generation during the dilution or mixing process is not yet well understood.

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Main Results: In this paper, we propose an integrated scheme for reducing waste droplets generated during dilution process on a DMF biochip. We analyze the inherent limitations of two earlier algorithms namely, bit-scanning (BS) [9], and dilution/mixing with reduced wastage (DMRW) [10] from the perspective of waste generation. Based on certain combinatorial properties of discrete dilution process, we propose an improved dilution/mixing algorithm (IDMA), and an integrated scheme for selecting the most efficient algorithm for waste reduction among BS, DMRW, and IDMA, given a concentration factor. Finally, an architectural layout of the chip for supporting the algorithm is presented.

II. Prior Work: Dilution of Fluids with DMF Biochips

DMF biochips typically work with discrete droplets on a uniform 2D-array of equi-sized electrodes, hence their volumes are always integral multiples 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 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.

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.

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, once two unit-volume droplets are mixed to get a new CF, 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 fewest mix/split steps with minimum number of waste droplet generation [11], [9], [10].

Recently, a mixing algorithm based on bit-scanning (BS) method has been proposed by Thies et al. [9] for mixing two or more fluids (sample/reagent) at any given ratio considering $(1:1)$ mixing model. In the special case of diluting a sample, this method first represents the target CF as an n-bit binary string depending on the precision level and then scans the bits from right-to-left to decide upon the sequence of mix/split steps. This method has the advantage that no storage of droplets with earlier intermediate CFs is required; only the current droplet along with the initial sample or the buffer is required for the next step. However, it produces one waste droplet at each mix/split step except the last one and it does not have any control over the waste droplet generation while diluting a fluid sample/reagent. It completes the dilution process in at most n mix-split steps to warranty a precision of $\frac{1}{2^n}$ in the target CF. Griffith et al. [11] described another algorithm based on a different strategy. It makes use of intermediate droplets but waste management issues were not addressed. A recently proposed scheme namely DMRW (dilution/mixing with reduced wastage) algorithm [10] can however, reduce the number of waste droplets compared to the BS method for many cases of the target CFs by properly reusing the intermediate CF droplets.

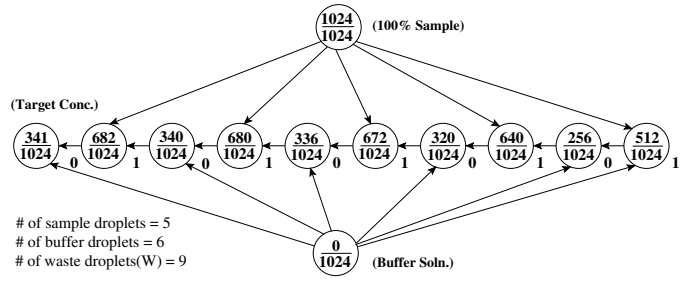


Fig. 2. Mix/split steps obtained by BS method [9] for the target CF $C_t=\frac{341}{1024}(\equiv 0.0101010101_2)$.

III. Proposed Scheme for Dilution and Mixing

A. Motivation of the Work

We have analyzed the performance of two dilution algorithms namely, BS [9] and DMRW [10] for several CFs over the range $\frac{1}{1024}$ to $\frac{1023}{1024}$ starting from a sample (100% conc.) and a buffer solution (0% conc.). The BS method always uses either sample or buffer solution at each $(1:1)$-mix/split step to mix with the current droplet. Thus, it uses only one droplet of intermediate CF only once and produces one waste droplet at each mix-split step (excepting the last one). For example, the mix/split procedure for the target CF $C_t=\frac{341}{1024}$ for the BS method is illustrated in Fig. 2, where the number of waste droplets is nine.

In DMRW, to achieve a target CF, the droplets of intermediate CFs may be needed multiple times in the mix-split process. Thus, to meet the demand of intermediate CF values, these droplets are to be produced starting from the given supply of sample and buffer. Although DMRW can reduce wastage in many cases, it may increase the total number of $(1:1)$-mix/split steps compared to BS method. To alleviate this problem, a layout architecture with a rotary mixer has been used for performing $(k:k)$ mixing $(k\geq 1)$ in one step [10]. The sequence mix/split steps for DMRW can be conveniently depicted using a *mix/split directed graph (digraph)* as shown in Fig. 3. A *digraph*, $M=(V,E)$, is a directed graph represented by a set of nodes V and set of directed edges, $E=\{\{v_i,v_j\}|v_i,v_j\in V\}$ (each edge denoted as an ordered pair of nodes). For example, the mix/split sequences of target CF values $C_t=\frac{127}{1024}$, $C_t=\frac{513}{1024}$, $C_t=\frac{16}{1024}$ and $C_t=\frac{341}{1024}$ are shown in Fig. 3.

We observe that the outdegree (skew) of a node representing an intermediate CF value in the digraph has a strong impact on waste droplet generation as well as on the number of $(1:1)$-

mix/split steps. This follows from the fact that in each $(1:1)$-mix/split step, two droplets are produced and therefore, the demand on this node as well as the wastage is optimized when both these droplets are used for subsequent mixing, and no more than two droplets are needed.

In case of $C_t = \frac{127}{1024}$, the node with $CF = \frac{128}{1024}$ is demanded multiple times (outdegree $= 7$). We call such a node having an outdegree (≥ 3) as a skew node s. For $CF = \frac{128}{1024}$ node, the skew value $SK(s) = 7$. Similarly, for $C_t = \frac{513}{1024}$, $CF = \frac{512}{1024}$ has $SK(s) = 7$ and for $C_t = \frac{16}{1024}$, $CF = C_l$ has skew $SK(s) = 6$ (see Fig. 3). However, for $C_t = \frac{341}{1024}$, there is no skew node and only one droplet is waste (which is the minimum). It may also be noted that the same mix/split sequence for target $CF = \frac{16}{1024}$ is obtained by both BS and DMRW.

In $(1:1)$ mixing model, mixing of two unit-volume droplets of CFs X and Y (denoted as $mix(X,Y)$), yields a mixture of $CF = \frac{X+Y}{2}$. Thus, to limit the error in target CF C_t by $\frac{1}{2^n}$, one needs a sequence of at most n mix/split steps. So, for the convenience of executing a dilution algorithm, each CF value, which always lies within 0 and 1 is approximated as a rational number with 2^n in the denominator. In order to describe the proposed scheme for minimizing both the waste and number of $(1:1)$-mix/split steps, we first explain the algorithm DMRW [10]. The notations used in the algorithm are shown in Table I.

TABLE I
NOTATIONS USED IN THIS PAPER.

M	Digraph corresponding to the sequence of *mix_split* steps obtained by DMRW for the given inputs C_l, C_h, C_t and n
M'	Modified digraph corresponding to the modified sequence of *mix_split* steps obtained by IDMA
$N(C_i)$	Node in a digraph corresponding $CF = C_i$
$CF(i)$	CF at the node i in a digraph
$LP(i)$	Left parent node of node i in a digraph
$RP(i)$	Right parent node of node i in a digraph
$O(i)$	Outdegree of a node i in a digraph
$I_1(i), I_2(i)$	Left and right indegree of a node i in a digraph
$U(i)$	Number of used or demanded droplets of the CF denoted by node i in a digraph
$D(i)$	Number of droplet produced of the CF denoted by node i in a digraph to satisfy the demand
$W(i)$	Number of droplets of the CF denoted by node i wasted in the *mix_split* sequence of a digraph
W	Total number of waste droplets in the *mix_split* sequence of a digraph
$Q(1:1)$	Total number of $(1:1)$-mixsplit steps in a digraph, e.g., one $(k:k)$-mix/split step requires k $(1:1)$-mix/split steps
$SK(i)$	skew or outdegree ($\geq$3) of a node i in a digraph

We use two steps called the *Preprocessing Step* and the *Performance Computation Step* given as follows:

Preprocessing Step:

1. Represent C_l as $\frac{a}{2^n}$ and C_h as $\frac{b}{2^n}$ for the precision level n, where a, b and n are positive integers and $0 \leq a < b \leq 2^n$.

2. Approximate C_t as $\frac{c}{2^n}$, where c is a positive integer and $0 \leq a < c < b \leq 2^n$.

3. Set two input CFs as $C_{in1} = C_l$ and $C_{in2} = C_h$.

Performance Computation Step of a Digraph:

1. Set outdegree of $N(C_t)$, i.e., $O(N(C_t)) = 2$, so two indegrees of $N(C_t)$ are $I_1(N(C_t)) = 1$ and $I_2(N(C_t)) = 1$.

2. Backtrace the digraph starting from node $N(C_t)$. For each node i, compute $O(i)$ from the indegrees of its children. Compute two indegrees of node i as $I_1(i) = I_2(i) = \lceil O(i)/2 \rceil$.

Input: C_{in1}, C_{in2}, C_t, n
Output: A digraph, M
Set $L = C_{in1}$ and $R = C_{in2}$;
Compute $X = \frac{L+R}{2}$ and Set $Q(k:k) = 1$;
Set $M = \{\{N(L), N(R), N(X)\}, \{\{N(L), N(X)\}, \{N(R), N(X)\}\}\}$;
Calculate *error* in CF as $|X - C_t|$;
while $error \geq \frac{1}{2^n}$ **do**
  if $X < C_t$ **then**
    Set $L = X$;
  end
  else
    Set $R = X$;
  end
  Compute $X = \frac{L+R}{2}$ and Set $Q(k:k) = Q(k:k) + 1$;
  Set $M = M \cup \{\{N(X)\}, \{\{N(L), N(X)\}, \{N(R), N(X)\}\}\}$;
  Calculate *error* in CF as $|X - C_t|$;
end
Obtain M with $Q(k:k)$ sequential mix/split steps;

Algorithm 1: DMRW Algorithm [10].

3. For each node i compute $D(i) = I_1(i) + I_2(i)$, $U(i) = O(i)$ and $W(i) = D(i) - U(i)$, and hence $W = \sum_{all\ nodes} W(i)$.

4. Scan the digraph to compute $Q(1:1) = \sum_{all\ nodes} I_1(i)$.

B. A Motivating Example

Consider the target $CF = \frac{341}{1024}$, for which the $(1:1)$-mix/split steps are shown in the digraph of Fig. 3(d) obtained using the DMRW algorithm. Here, the number of waste droplets is the smallest (only one); this motivates us to analyze the underlying combinatorial properties hidden in the dilution process.

It is observed that, in the mix-split digraph, if the progression sequence towards the target CF alternates between the left(right) and right(left) arms, then the outdegree of each node can never exceed two, and therefore, the waste is optimized and no additional demand of intermediate CFs is created. Our analysis reveals that such an alternating mix-split sequence is possible resulting in two target droplets of CF C_i and with only one waste droplet, starting from two boundary CFs $C_l = \frac{a}{2^n}$ and $C_h = \frac{b}{2^n}$ after i $(1:1)$ mix-split steps, if and only if:

$$C_i = \frac{1}{3}\left[\left(2a + \frac{(-1)^{i+1}}{2^{i+1}}a\right) + \left(b + \frac{(-1)^i}{2^{i+1}}b\right)\right] \tag{1}$$

where, $i = 0$ to $\left(\left\lfloor \log_2(b-a) \right\rfloor - 1\right)$. Thus, waste is optimized when C_t is nearly one-third or two-third of the interval $(b-a)$.

If $C_l = 0$ (buffer solution with 0% conc.) and $C_h = \frac{2^n}{2^n}$ (sample fluid with 100% conc.), then Eqn. 1 reduces to:

$$C_i = \frac{1}{3}\left[\left(2^n + \frac{(-1)^i}{2^{i+1}}2^n\right)\right] \tag{2}$$

for $i = 0$ to $\left(\left\lfloor \log_2 2^n \right\rfloor - 1\right) = (n-1)$.

From Fig. 3(d), we get the $(1:1)$-mix/split steps for the target $CF = \frac{341}{1024}$ and it indicates the intermidate CFs 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 intermediate CF values can also be obtained from Eqn. 2, by setting $n = 10$ and putting values of i from 0 to 9, and for each of them waste W will be one.

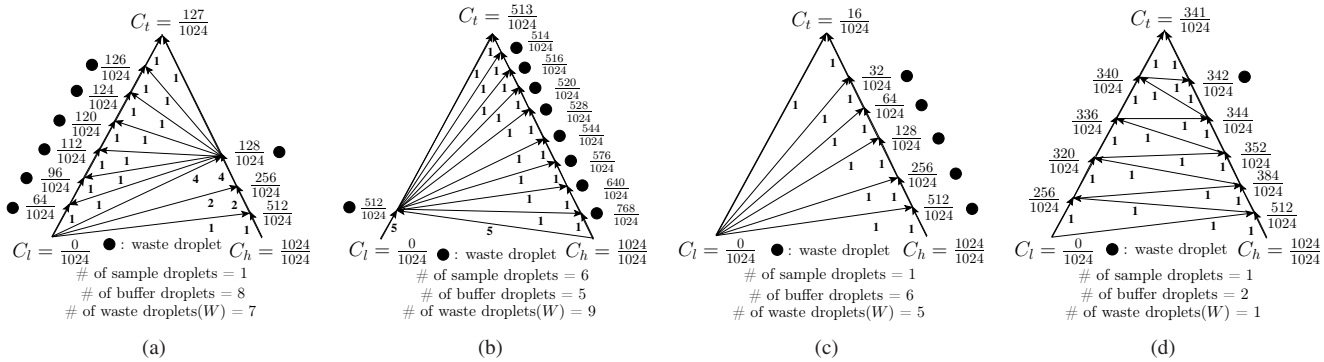


Fig. 3. Mix/split steps obtained by DMRW algorithm [10] for the target CF (a) $C_t = \frac{127}{1024}$, (b) $C_t = \frac{513}{1024}$, (c) $C_t = \frac{16}{1024}$ and (d) $C_t = \frac{341}{1024}$.

C. Improved Dilution/Mixing Algorithm (IDMA): A Heuristic

The above observation motivates us to further reduce waste droplets generated by DMRW scheme [10], by controlling the skew of an intermediate CFs node within the maximum error bound of $\frac{1}{2^n}$. However, it strongly depends on the given target CF in the range of two input CFs.

We propose a heuristic algorithm for reducing both the number (W) of waste droplets and the number ($Q(1:1)$) of $(1:1)$-mix/split steps to generate a given target CF. While there is a skew at any intermediate CF (other than two input CFs) in the mix/split sequence, we attempt to modify the bounding interval such that the target CF is nearly equal to the one-third or two-third of the modified interval. In order to achieve this, we find a new lower boundary CF or a new upper boundary CF depending on the relative distance of the target CF from the current lower and upper boundary CFs to neutralize the skew that appears first in the mix-split sequence. This simple heuristic applied on DMRW can significantly reduce both W and $Q(1:1)$. The proposed scheme IDMA is described as Algorithm 2.

The algorithm IDMA preserves the same precision of $\frac{1}{2^n}$ in C_t for n sequential $(1:1)$ mix-split steps as in DMRW. Since the first two 'for' loops in IDMA take $O(n)$ time, all the conditional (or 'if') statements need constant time, and execution of DMRW requires $O(n)$ time, the overall time complexity of IDMA remains $O(n)$.

D. Examples

Applying IDMA on the digraph obtained from DMRW for the two target CFs $C_t = \frac{127}{1024}$ and $C_t = \frac{513}{1024}$, we get two modified digraphs as shown in Fig. 4. Note that, in each case, both the number of waste droplets (W) and the number of $(1:1)$-mix/split steps have been reduced compared to DMRW, and W is reduced compared to that of the BS method as well. However, the digraph obtained from DMRW for the target CFs (such as $\frac{1}{1024}$ or $\frac{1023}{1024}$, etc.) has high skew on the two input CFs (such as 0% and 100% concentrations); for these cases, IDMA cannot improve W or $Q(1:1)$. Another extreme case (for the target CF $C_t = \frac{16}{1024}$), which IDMA cannot improve is shown in Fig. 3(c). Here, the skew node is at $CF = C_l = 0\%$ and the two methods BS and DMRW will produce the same mix/split sequence with the same W and $Q(1:1)$ values.

Input: C_l, C_h, C_t, n and a digraph obtained by DMRW, M
Output: A modified digraph, M'
foreach *Node i* **do**
  Compute outdegree or $SK(i)$;
end
Set $Flag = 0$;
foreach *Node i in M starting from* $N(C_l)$ *and* $N(C_h)$ **do**
  if $SK(i) \geq 3$ *and* $(CF(i) \neq C_l$ *or* $CF(i) \neq C_h)$ **then**
    Set $Flag = 1$ and $G =$ subgraph of M up to node i;
    Exit from **for** loop;
  end
end
if $Flag = 0$ **then**
  $M' = M$ and **Exit**;
end
if $CF(j) = 2^{n-1}$ **then**
  if $CF(j) < C_t$ **then**
    $L = CF(LP(j))$ and $R = \left[CF(j) + CF(RP(j))\right]/2$;
    Add $N(R)$ and two directed edges to G;
  end
  else
    $L = \left[CF(j) + CF(LP(j))\right]/2$ and $R = CF(RP(j))$;
    Add $N(L)$ and two directed edges to G;
  end
end
else
  if $CF(j) < C_t$ **then**
    $L = \left[CF(j) + CF(LP(j))\right]/2$ and $R = CF(RP(j))$;
    Add $N(L)$ and two directed edges to G;
  end
  else
    $L = CF(LP(j))$ and $R = \left[CF(j) + CF(RP(j))\right]/2$;
    Add $N(R)$ and two directed edges to G;
  end
end
Execute DMRW with inputs L, R, C_t and n and obtain a digraph H;
Obtain the modified digraph as $M' = G \cup H$;

Algorithm 2: Improved Dilution/Mixing Algorithm (IDMA).

IV. Simulation Results

A. Integrated Scheme for Dilution/Mixing

We now present an integrated scheme for dilution of a biosample or mixing of two samples/reagents on a DMF biochip that allows us to choose the best algorithm among BS, DMRW, and IDMA depending on the given value of the target CF, with waste minimization as the first criterion and the number of $(1:1)$ mix-split steps as the second objective. The scheme is described in the flowchart of Fig. 5.

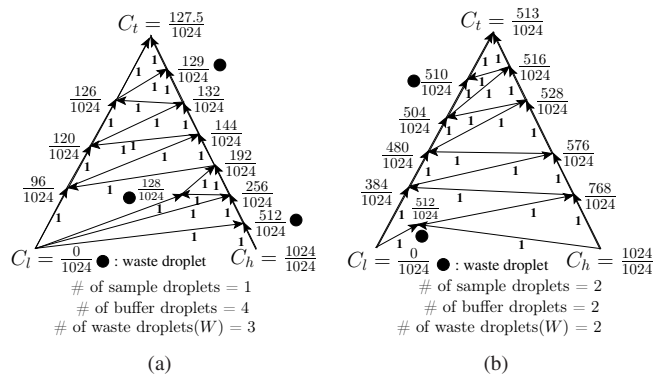


Fig. 4. Mix/split steps obtained by IDMA for (a) $C_t = \frac{127}{1024}$ and (b) $C_t = \frac{513}{1024}$.

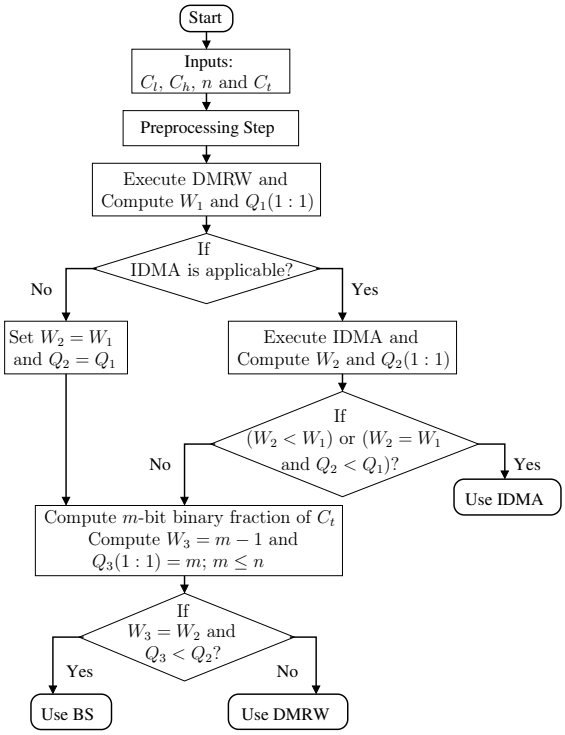


Fig. 5. Flowchart of the integrated scheme.

We have run experiments over 1023 target CF values from $\frac{1}{1024}$ to $\frac{1023}{1024}$, with the sample (100% conc. i.e., $C_h = \frac{1023}{1024}$) and buffer solution (0% conc. i.e., $C_l = \frac{0}{1024}$) as two inputs, using the three methods namely, BS [9], DMRW [10] and IDMA. Simulation results reveal that for a total of 334 target CFs, IDMA is best suitable for waste minimization (compared to BS [9] and DMRW [10]) along with reduced number of $(1:1)$-mix/split steps (compared to DMRW [10]). For a total of other 668 target CFs, DMRW is found to be the best for waste minimization. For the remaining 21 target CFs, neither DMRW nor IDMA can reduce waste compared to that generated by the BS method, and hence, it is better to use BS as it requires minimum number of $(1:1)$-mix/split steps, and it is simple to implement. The piechart shown in Fig. 6 reflects the results. To illustrate the selection process, we show a range of target CFs from $\frac{282}{1024}$ to $\frac{293}{1024}$ in Fig. 7.

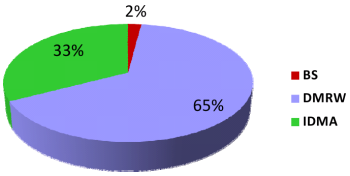


Fig. 6. Distribution of 1023 target CFs for waste minimization.

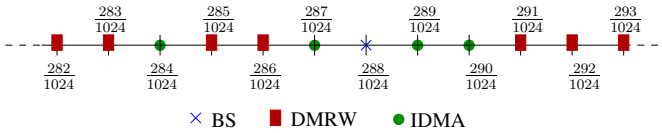


Fig. 7. Selection of the best method for different target CFs in the range of ($\frac{282}{1024}$ to $\frac{293}{1024}$) for waste minimization.

B. Comparative Study

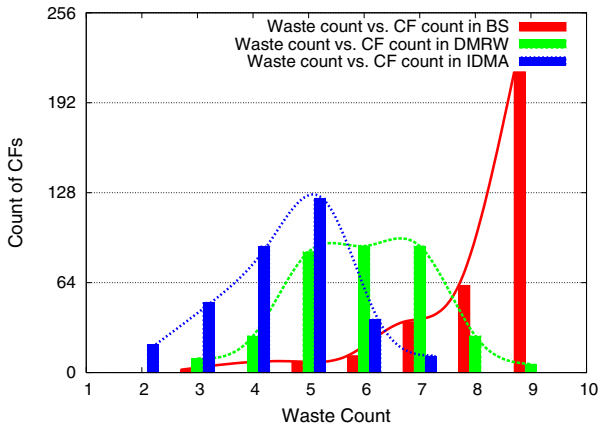
For some example target CFs, results of the above three methods are shown in Table II in terms of the number of waste droplets (W), and the number of $(1:1)$-mix/split steps ($Q(1:1)$). For target CFs $\frac{127}{1024}$, $\frac{513}{1024}$, $\frac{313}{1024}$ and $\frac{287}{1024}$ IDMA method will be used, since this provides best reduction of waste among the three methods, and $Q(1:1)$ is also reduced compared to the DMRW method. For CFs $\frac{16}{1024}$ and $\frac{341}{1024}$, IDMA is not executed, because the skew obtained in the digraph of DMRW for $C_t = \frac{16}{1024}$ is on the input CF and there is no skew at any intermediate node of the digraph for $C_t = \frac{341}{1024}$. So, the integrated scheme outputs the same digraph as obtained by DMRW. Finally, BS is chosen for the first one and DMRW for the second one, after comparing the W-values and $Q(1:1)$-values. Similarly, DMRW is to be used for $C_t = \frac{283}{1024}$ and BS for $C_t = \frac{288}{1024}$ as apparent from the results shown in the table.

Since IDMA performs well for 334 target CFs compared to BS and DMRW, we report only these CFs to judge their performances. For each of the 334 target CFs where IDMA is to be used, the values of W and $Q(1:1)$ are computed after applying three methods. A comparative result of the number of waste droplet generation in the dilution process for those target CFs versus the count of CFs is shown in the histogram plots (Fig. 8(a)). It is observed that IDMA reduces the waste significantly compared to BS and DMRW techniques. Comparative results on the total number of $(1:1)$-mix/split steps required in the dilution process for these target CFs versus the count of CFs are shown in the histogram plots (Fig. 8(b)). We observe that IDMA can further reduce the $Q(1:1)$-value compared to DMRW for several target CFs over this range. Similar to DMRW, IDMA has a trade-off between

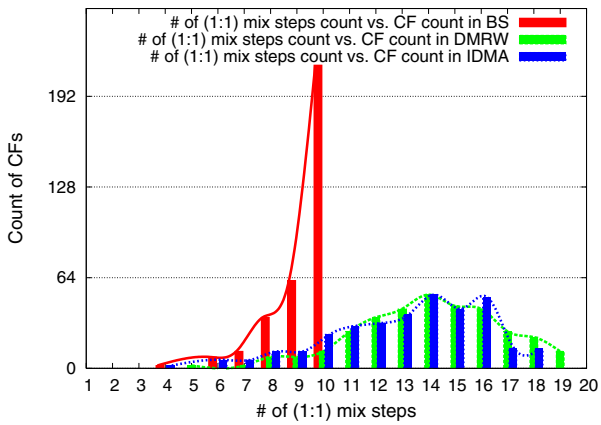
TABLE II
COMPARATIVE RESULTS OF THREE METHODS FOR SOME EXAMPLE CFS.

Target CF (≡binary)	BS		DMRW		IDMA	
	W	$Q(1:1)$	W	$Q(1:1)$	W	$Q(1:1)$
16/1024 ($\equiv 0.000001_2$)	5	6	5	6	*	*
127/1024 ($\equiv 0.0001111111_2$)	9	10	7	14	3	11
513/1024 ($\equiv 0.1000000001_2$)	9	10	9	14	2	10
341/1024 ($\equiv 0.0101010101_2$)	9	10	1	10	*	*
313/1024 ($\equiv 0.0100111001_2$)	9	10	7	18	5	15
283/1024 ($\equiv 0.0100011011_2$)	9	10	5	14	5	16
287/1024 ($\equiv 0.0100011111_2$)	9	10	8	18	5	13
288/1024 ($\equiv 0.01001_2$)	4	5	4	7	4	14

* IDMA is not performed for this CF. Integrated scheme uses BS or DMRW for it.



(a)



(b)

Fig. 8. Histograms of three methods for 334 target CFs. (a) Distribution W-values and (b) Distribution of $Q(1:1)$-values with the count of CFs.

reduction of waste droplets and increase in the number of $(1:1)$-mixing steps.

V. A Layout for Integrated Scheme

Implementation of DMF rotary mixers has been demonstrated earlier [12], [10]. For the DMRW method, it is reported that $(k:k)$-mixing is required for a maximum value of $k=5$ over the range of 1023 discrete target CFs between $\frac{1}{1024}$ and $\frac{1023}{1024}$ to expedite the dilution process [10]. We observe that in the proposed integrated scheme, when IDMA is chosen for 334 target CFs, k is at most 3; for the 668 target CFs chosen by DMRW, k is found to be at most 4; for the remaining 21 cases where BS is chosen, $k=1$. Hence, to implement the integrated dilution-on-chip scheme, an architectural layout of electrodes with a rotary mixer can be used for IDMA and DMRW as shown in [10]. A portion of the same layout can be used as a 3-electrode array mixer to perform $(1:1)$ mixing needed for BS. This array mixer uses merge-and-split operation for mixing two droplets [13]. The overall layout is shown in Fig. 9.

VI. Conclusions

In this paper, we present an integrated scheme for reducing the number of waste droplets or mixing steps during the

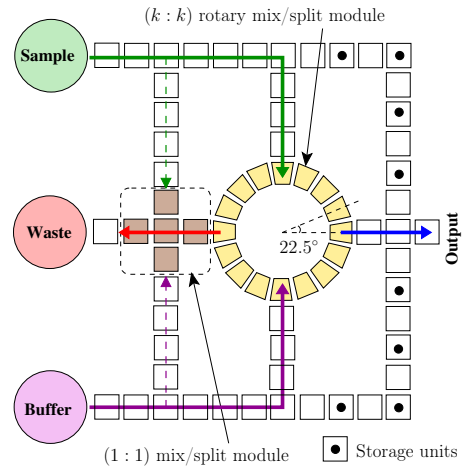


Fig. 9. Layout of electrodes for integrated scheme of dilution.

dilution/mixing of two samples/reagents on a DMF biochip. Simulation results elaborate the efficacy of the proposed method. A befitting layout architecture that supports this scheme on-chip is also designed. Several open problems stem out of this study. One of them is to design a mixing algorithm using $(k:\ell)$-mixing model for $k \neq \ell$. For example, to achieve target $CF = \frac{341}{1024}$ from 100% sample and 0% buffer, for which ten $(1:1)$ mix-split steps are required by BS, DMRW, or IDMA, just one $(2:1)$-mixing step is sufficient (i.e., when two droplets of the buffer and one droplet of the sample are combined).

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