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Simulation-based method for optimum microfluidic sample dilution using weighted mix-split of droplets

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Abstract: Digital microfluidics has recently emerged as an effective technology in providing inexpensive but reliable solutions to various biomedical and healthcare applications. On-chip dilution of a fluid sample to achieve a desired concentration is an important problem in the context of droplet-based microfluidic systems. Existing dilution algorithms deploy a sequence of balanced mix-split steps, where two unit-volume droplets of different concentrations are mixed, followed by a balanced-split operation to obtain two equal-sized droplets. In this study, the authors study the problem of generating dilutions using a combination of (1 : 1) and (1:2) mix/split operations, called weighted dilution (WD), and present a layout architecture to implement such WD-steps. The authors also describe a simulation based method to find the optimal mix-split steps for generating a dilution under various criteria such as minimisation of waste, sample, or buffer droplets. The sequences can be stored in a look-up table *a priori*, and used later in real time for fast generation of actuation sequences. Compared with the balanced (1:1) model, the proposed WD scheme reduces the number of mix-split steps by around 22%, and the number of waste droplets, by 18%.

1 Introduction

Advances in microfluidics and microfabrication technologies find many versatile applications to molecular biology, DNA analysis, stem-cell manipulation, virology, point-of-care experiments with BioPen, self-assembly of hydrogels, and for building 3D devices [1–6]. In recent times, lab-on-chips based on digital microfluidic (DMF) technology, have enabled the automation of various biochemical protocols accurately on chip, at a very low cost [7–16]. The phenomenal growth perceived in this technology has opened up a plethora of algorithmic challenges such as synthesis of protocols, design of chip-layout, and path-planning for droplet routing on a DMF biochip [7]. One important problem in algorithmic microfluidics, which addresses the above-mentioned optimisation problems, is sample preparation, that is, to design efficient dilution or mixing algorithms for fluid droplets. The objective is to prepare certain dilution of a fluid sample, or a mixture of several reagents in a certain ratio, while minimising the number of mix-split steps, the usage of input droplets, or waste production [8–10, 14–16].

The dilution and mixing of fluids are two fundamental steps of any chemical assay, and a sequence of (1:1) mixing followed by a balanced splitting is used in sample preparation with DMF s [8–10]. In such a (1:1) mix/split operation, two unit-volume droplets of a sample that are diluted with the same diluent, are mixed together and then split into two unit-volume droplets. The concentration of the resulting droplets is the arithmetic mean of those of the two parent droplets used for mixing.

Weighted dilution (WD) [17, 18] is based on a more powerful mixing model (k:l), where a number of droplets (say k) of one concentration can be mixed with l droplets of another concentration to achieve a desired concentration of a sample, where k, $l \geq 1$. It has been observed that for a given accuracy of target concentration factor (CF), WD requires fewer mix-spit steps compared with the use of (1:1) model alone. This in turn, often reduces the number of waste droplets [19]. However, a weighted mix-split step is more expensive than the baseline (1:1) mix-split step because it is not only more difficult to implement on-chip but takes longer time to perform a mix or a split operation.

In this paper, we restrict the weighted steps only to (1:2) or (2:1) mixing and present how they can be used together with (1:1) mixing to generate droplets of desired concentration. Note that if we mix two solutions, one being the buffer with concentration 0/3 (0%) and the other being a sample with concentration 3/3 (100%) in (2:1) ratio, then we will get a solution with concentration equal to

$$\frac{0/3 \times 2 + 3/3 \times 1}{3} = \frac{1}{3}(33.33\%)$$

of the original sample. Similarly, mixing them in (1:2) ratio, will give a final concentration of 2/3. Hence for denominator 3, we can generate concentrations with any integral numerator lying between 0 and 3. We can generate all fractional concentrations

$$\frac{1}{9}, \frac{2}{9}, \cdots, \frac{8}{9},$$

using only two (1:2) or (2:1) mixing steps [19].

More generally, it can be proved by induction that using a maximum of r (1:2) or (2:1) mixing steps, it is possible to generate any integral numerator between 0 and 3^r with denominator 3^r. Note that in the above case, generation of some of the fractions within r steps is not always trivial. In fact, in some cases, feasible solutions do not exist for all intermediate fractions. As for example, instead of (1:2) type of mixings, if we allowed only (1:3) or (3:1) type of mixings, we could generate the fractions 1/4 and 3/4, but we cannot generate 2/4 = 1/2 at all in this setting. One can also use larger ratios such as (1:3), (1:4), or (2:3), but mixing algorithms based on such higher ratios will be more complicated and they are likely to produce more waste droplets compared with the use of small-ratio mixers. Moreover, implementation of such mixers will be difficult; mixing time also increases proportionately for high-ratio mixers. Efficient implementation of mixing algorithms with larger ratios thus needs further investigation. Hence, in this work, we have considered only (1:1) or (1:2) ratio, which improves the performance compared with the use of baseline ratio (1:1). Compared with the

balanced (1:1) model, the proposed WD scheme reduces the number of mix-split steps by around 22%, and the number of waste droplets, by 18%.

We have also assumed that every split operation is ideal, that is, a 2X-volume droplet can be perfectly split into two 1X-volume droplets, and from a 3X-volume droplet, an 1X-volume droplet can be separated without any volumetric error. However, in practice, a split operation is often not ideal, and hence, it may have an impact on the target concentration. To achieve error-tolerance in the presence of such imperfect fluidic operations, a cyberphysical system with sensor feedback mechanism will be required [20].

In Section 2, we briefly review prior art, and present our formulation in Section 3. A proof that any fraction can be generated using (1:2)/(2:1) mix-split steps is presented in Section 4. The WD scheme and experimental results are discussed in Section 5. The proposed WD scheme, experimental results, and performance comparison with the baseline ratio (1:1) in terms of mix-split steps, reactant usage, and waste droplets, are presented in Section 5. Finally, in Section 6, we conclude our work.

2 Prior work

Sample preparation is an important step in any biochemical protocol, and several algorithms are known for automating them on a DMF lab-on-a-chip [8–13]. However, all prior approaches are based on the usage of only (1:1) mix-split operations, and they are incapable of handing any kind of weighted mix-split steps. On-chip dilution of a sample on a DMF biochip involves several mix-split steps, which often suffer from the inaccuracies caused by unbalanced splitting of micro-fluid droplets. Moreover, error minimisation is highly desirable because of the limited availability of the stock solutions and costly reagents. Bera *et al.* analysed the performance [19] of two dilution algorithms, Min-Mix or TwoWayMix [14] and dilution and mixing with reduced wastage [15, 16] in the presence of volumetric errors that may occur during the splitting process.

3 Problem formulation

To generate a target fraction (C_t), we propose to start the WD algorithm with two initial CFs (CFs). At every mix-split step, the algorithm requires two CFs, called the boundary CFs, one lower and one higher than the C_t. If C_t is inclined more towards the lower CF, then the lower CF is mixed more to generate the intermediate CF, that is, by mixing the lower and higher CFs in (2:1) ratio, otherwise mixed in (1:2) ratio. Using the proposed scheme, we can achieve any fraction (C_t) on top of a pre-computed denominator. The denominator depends on the maximum number of (1:1) and (1:2) or (2:1) steps allowed. As for example, any fraction from 1 to 17 can be generated with a chosen denominator ($2^1 \times 3^2 = 18$) by using at most one balanced and two weighted steps. A target concentration C_t (e.g. 9/18) may be achieved either in one step of (1:1) mixing or by using a maximum of three mix-split steps (e.g. 1/18).

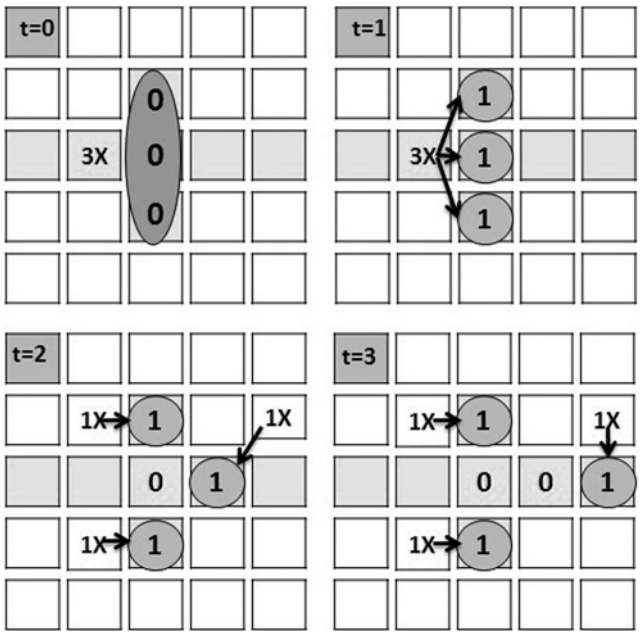

Fig. 1 *Splitting of one 3X droplet into three 1X droplets on a 2D rectangular layout*

In (1:2) weighted scheme, the mixing of three droplets can be accomplished either by a conventional mixer [17], or by using a pathway mixer where the droplets are mixed to each other while being navigated along a path or along a linear arrangement of electrodes. Consider Fig. 1. A '0' mark on any electrode denotes that it is connected to the ground and on the other hand, a '1' mark denotes that it is connected to a high actuation voltage. The splitting of one three-unit volume (3X) droplet into three unit-volume (1X) droplets can be implemented by instantiating a T-type orthogonal pattern on a 2D regular rectangular electrode array. Consider a 3X droplet at time $t = 0$; at time $t = 1$, we apply the control voltage to all the three electrodes to begin the splitting process. By varying the patterns of control voltage activation, the 1X droplet is moved right at time $t = 2$. At time $t = 3$, again the same 1X droplet is shifted further right. Such $3X \rightarrow 1X$ mix-split process can be performed more accurately using a customised electrode pattern layout where the three arms are oriented mutually at 120° apart (Fig. 2). In this architecture, during the split operation, the pulling force along the right arm counter-balances that on the left, which is the sum of horizontal components of the forces along the other two arms. This tends to minimise the volumetric imbalance during splitting of a 3X droplet into three 1X droplets.

The fraction or sequence generation problem can now be formally stated as follows. Given the maximum number of balanced steps b and weighted steps w,

(i) Compute the denominator $D = 2^b \times 3^w$ and maximum number of total steps $t = b + w$.
(ii) Initial lower numerator = 0 (buffer) and initial upper numerator = D (sample).
(iii) Goal is to achieve all the fractions with numerators within d1 to $D - 1$ using (1:1) and/or (1:2)/(2:1) mixing steps.
(iv) While generating a particular fraction, we try to minimise one or more of the following criteria, that is, the number of (a) total mixing steps, (b) weighted steps, (c) waste (d) sample and (e) buffer droplets.

4 Generation of any fraction using (1:1) and (1:2)/(2:1) mix-model

Suppose we are mixing p (1:1) steps and q ((1:2) or (2:1)) steps. Hence denominator is $2^p \times 3^q$. We now present a method to show

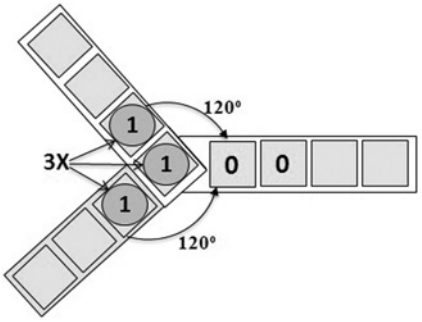

Fig. 2 *Accurate splitting of a 3X droplet into three 1X droplets using a customised mix-split unit*

that all the fractions with numerators –varying from 1 to $(2^p \times 3^q - 1)$ can be generated using at most $(p+q)$ steps. We show below that there exists at least one way for generating each fraction.

First, we use up the balanced steps as many times as required. After a maximum of p balanced steps, we can generate an interval of size 3^q and then we can generate any fraction in an interval of 3^q using q number of (2:1) or (1:2) mixing steps. For illustration, let $p=3$ and $q=2$, then denominator $=8\times 9=72$. To get a fraction of 36/72, a single 1:1 step is necessary. To get 18/72, a second 1:1 step with boundaries 0/72 and 36/72 is required. However, for 22/72 we use the 3rd 1:1 step with boundaries 18/72 and 36/72 to first get the fraction 27/72. Then we will have two boundary concentrations of 18/72 and 27/72 with an interval of $9=3^q$ (where $q=2$) in the numerator.

Lemma 1: We can generate all the fractions with distinct numerators ranging from 1 to 3^q-1 and denominator 3^q, using a maximum of q weighted mixing steps.

Proof: We will show the above result by proving a stronger claim using induction. To show that we can generate 3^q-1 distinct fractions at equal intervals between 2 given fractions using a maximum of q weighted mixing steps.

Base step: When $q=1$, using the smaller fraction (say 0/3) and larger fraction (say 3/3) as the boundary concentrations, mix 0 and 3 using (1:2) mix-model to get numerator 2 (i.e. 2/3 concentration) or mix 0 and 3 using (2:1) dilution to get numerator 1 (i.e. 1/3 concentration). Note that it holds true for any generalised numerators. If we used x and $(x+3)$ instead of 0 and 3 respectively, then $(2x+(x+3))/3$ would have given us $(x+1)$ and $(x+2(x+3))/3$ would have given us $(x+2)$. Hence from x/y and $(x+3)/y$, we would successfully generate $(x+1)/y$ and $(x+2)/y$ in just 1 weighted mixing step.

Induction hypothesis: We can generate $3^{q-1}-1$ distinct fractions at equal intervals between 2 given fractions using a maximum of $q-1$ weighted mixing steps.

Induction step: Suppose the numerators of the lower and upper boundaries are L and U respectively. Specifically in our case $U-L=3^q$.

After using just one weighted step, we have

Case 1: If we use (1:2) mixing of L and U, we will get

$$U' = \frac{(L+2U)}{3} = L + 2\times 3^{q-1}$$

(by substituting $U=L+3^q$).
Case 2: If we use (2:1) mixing of L and U, we will get

$$L' = \frac{(2L+U)}{3} = L + 3^{q-1}$$

(by substituting U again).

Note that $L'-L=U'-L'=U-U'$. Hence L' and U' divide the whole range into three equal parts. In our case, L and U are separated by 3^q and hence each part is equal to 3^{q-1}. If now the target concentration lies

(a) In the upper half – Use Case 1 above and then use the induction hypothesis between U' and U.
(b) In the lower half – Use Case 2 above and use the induction hypothesis between L and L'.
(c) In the middle half – This is the non-trivial part. Note that if we had created both L' and U', we could have used the induction hypothesis easily to get any numerator in this interval also. However, using only 1 weighted step, we could not have created both L' and U' and hence we would have used $2+(q-1)=q+1$ steps in total, which is not intended. Hence we need to use a trick. Using L and U' as the two boundaries, and the induction hypothesis we can generate all the numerators from L to U' at an arithmetic progression of 2. Similarly, using L' and U as the two boundaries, and the induction hypothesis we can generate all the numerators from L' to U at an arithmetic progression of 2. Together we can exhaust all the numerators from L' to U'. Hence the proof.

□

Example 1: Take $q=2$. Starting fractions $L=0/9$ and $U=9/9$. Now after the 1st step we can generate $L'=3/9$ or $U'=6/9$.

(i) Mix L and L' to generate 1/9 and 2/9.
(ii) Mix U' and U to generate 7/9 and 8/9.
(iii) Mix L and U' to generate 2/9 and 4/9.
(iv) Mix L' and U to generate 5/9 and 7/9.

Note that all fractions were generated for $q=2$.

Example 2: Now for $q=3$, let, $L=0/27$, $L'=9/27$, $U'=18/27$ and $U=27/27$.

We can use Example 1 to generate 1/27 to 8/27 and 19/27 to 26/27. The difficult numerators are 10, 11, 12, 13, 14, 15, 16 and 17. Out of these 10, 12, 14 and 16, that is, the even ones can be generated using 0/27 and 18/27 as boundaries, whereas the odd ones can be generated by using 9/27 and 27/27 as boundaries.

5 Proposed scheme

Note that for optimising every different criterion out of those five stated in the problem formulation (Section 3), we may need a different sequence of weighted mix-split steps. Moreover, for some fractions it may be possible to optimise all the above criteria simultaneously, but not for all. Another important observation is that even for the balanced dilution method, the maximum number of mix-split steps that suffices for all practical purposes is around 10, that is, having a denominator of $2^{10}=1024$ in the CF is sufficiently accurate. Considering the above facts, we now propose to employ a method of exhaustive simulation for generating the optimal sequence of steps for every criterion, which though exponential, makes perfect sense as the number of steps is small. Our simulation result demonstrates that the proposed method is useful in implementing on-chip dilution efficiently.

We adopt a look-up table method to search for an optimal mix-split sequence required to generate a given target concentration. The table is constructed beforehand off-line by exhaustive enumeration as stated earlier; at the time of protocol realisation, when a target concentration is requested, the best sequence is found in real-time immediately. Note that this can be done entirely offline just by knowing b and w. When the actual

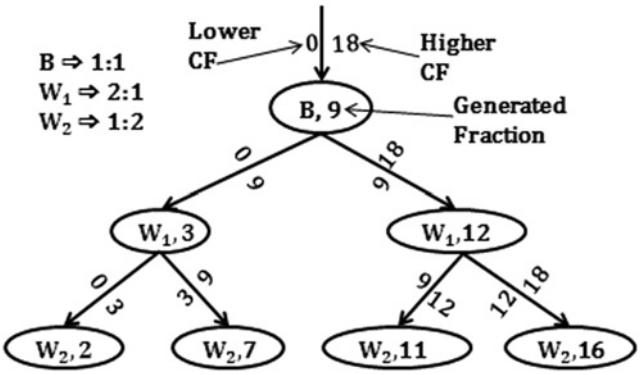

Fig. 3 *Generation of 7 different fractions from sequence BW_1W_2*

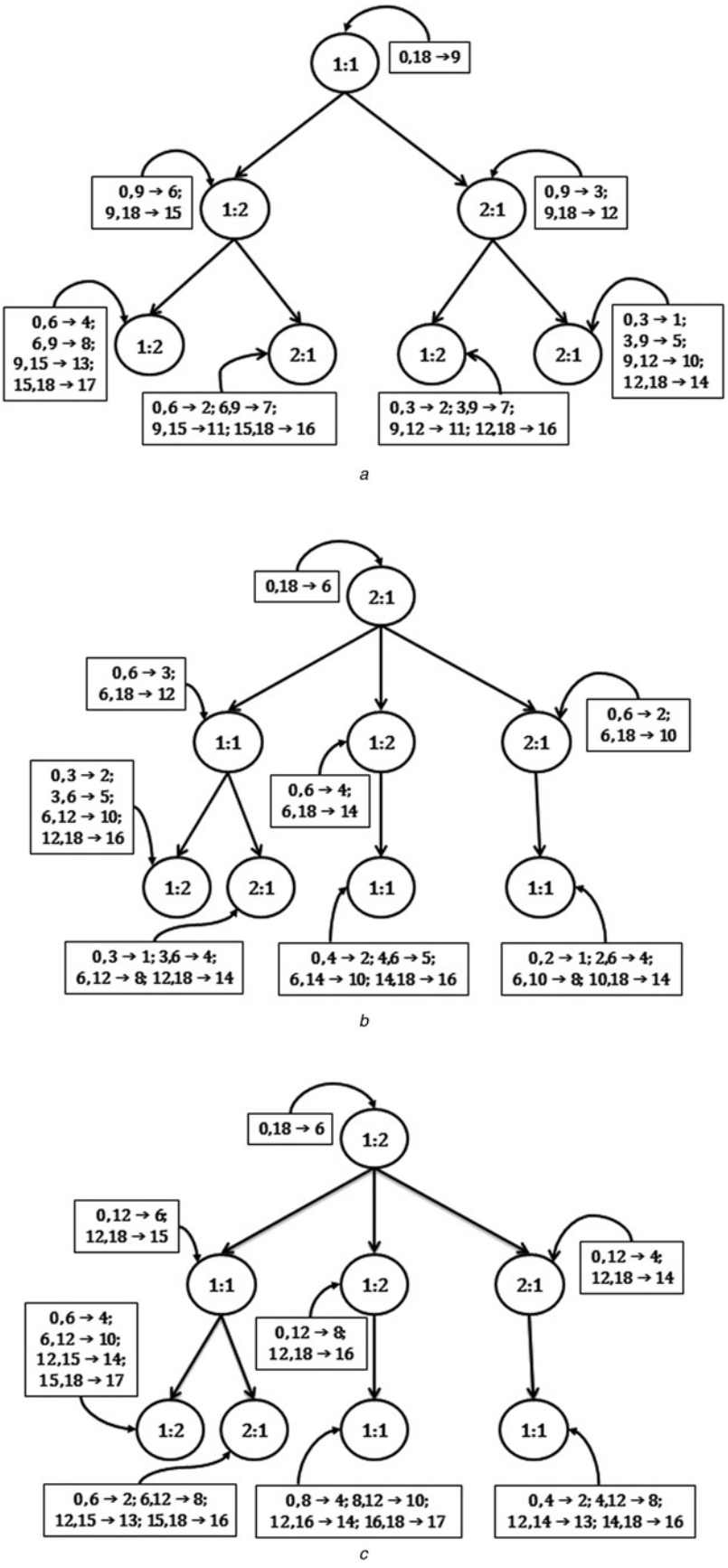

Fig. 4 *Unique ways of generating fractions*

a 21 unique step-sequences starting with (1:1)
b 23 unique step-sequences starting with (2:1)
c 23 unique step-sequences starting with (1:2)

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Table 1 Step sequences for fraction generation with $D = 18$

Target	Frequency of mix-split sequences	Opt. steps	Wgt. steps	Waste	Input sample	Input buffer	Required mix-split sequence		
							Step 1	Step 2	Step 3
1	3	3	2	3	1	5	(2:1)-0-18-6	(1:1)-0-6-3	(2:1)-0-3-1
2	7	2	2	2	1	4	(2:1)-0-18-6	(2:1)-0-6-2	
3	2	2	1	1	1	3	(1:1)-0-18-9	(2:1)-0-9-3	
4	7	2	2	1	1	3	(2:1)-0-18-6	(1:2)-0-6-4	
5	3	3	2	1	1	3	(2:1)-0-18-6	(1:1)-0-6-3	(1:2)-3-6-5
6	3	1	1	0	1	2	(2:1)-0-18-6		
7	2	3	2	2	2	3	(1:1)-0-18-9	(1:2)-0-9-6	(2:1)-6-9-7
8	6	2	2	1	2	2	(1:2)-0-18-12	(1:2)-0-12-8	
9	1	1	0	0	1	1	(1:1)-0-18-9		
10	6	2	2	1	2	2	(2:1)-0-18-6	(2:1)-6-18-10	
11	2	3	2	2	3	2	(1:1)-0-18-9	(2:1)-9-18-12	(1:2)-9-12-11
12	3	1	1	0	2	1	(1:2)-0-18-12		
13	3	3	2	1	3	1	(1:1)-0-18-9	(1:2)-9-18-15	(1:2)-9-15-13
14	7	2	2	1	3	1	(1:2)-0-18-12	(2:1)-12-18-14	
15	2	2	1	1	3	1	(1:1)-0-18-9	(1:2)-9-18-15	
16	7	2	2	2	4	1	(1:2)-0-18-12	(1:2)-12-18-16	
17	3	3	2	3	5	1	(1:2)-0-18-12	(1:1)-12-18-15	(1:2)-15-18-17

assays will run on a biochip, they can use the best sequence to generate a particular fraction by reading the look-up table, losing no time for computation. Such small look-up tables can be loaded even in embedded systems with very little memory overhead.

5.1 Small example

Let us consider a small example with at most one balanced (B) and two weighted (W) steps. We note the following parameters and facts – (i) $b = 1$, $w = 2$, $t = b + w = 1 + 2 = 3$; (ii) Denominator $D = 2^b \times 3^w = 2^1 \times 3^2 = 18$; (iii) Number of ways the steps can be executed is three (BWW, WBW, WWB); (iv) each weighted step may be (2:1) (W_1) or (1:2) (W_2). Hence each of the above three sequences may be written in $2 \times 2 = 4$ different ways giving 12 permutations in total. Now, each of these three elements (B, W_1 or W_2) represents a mix-split step by which a new intermediate fraction will be generated. Hence the range of fractions designated by the two initial concentrations used for mixing will now be divided into two distinct ranges by the new fraction. For each of the 12 sequences, seven new fractions can be generated that can be represented using a complete binary tree (CBT) having three levels, as shown in Fig. 3. A CBT with three levels will have $2^3 - 1 = 7$ nodes, and hence a total of $12 \times 7 = 84$ different ways of generating fractions are counted above. In our simulation method we follow the above combinatorial method to generate the different fractions.

Lemma 2: For a maximum of b balanced steps and w weighted steps, the upper bound on the total number of ways to generate fractions is given by

$$\binom{b+w}{w} \times 2^w \times (2^{b+w} - 1).$$

Proof: Note that the Bs and Ws can be written in

$$\binom{b+w}{w}$$

ways. Each of the Ws may be written in two ways. Hence the total number of sequences is actually

$$\binom{b+w}{w} \times 2^w.$$

Finally, for each sequence of length $b + w$, the number of ways fractions can be generated is equal to the number of nodes present in a CBT with height $b + w - 1$, which is $(2^{b+w} - 1)$. Hence the result. □

Note that not all these fractions generated above are unique and obviously the same fraction can be generated in multiple ways. However, not all the ways to generate fractions counted by the above lemma are unique. For example, if in Fig. 3, we consider the sequence BW_2W_1 instead, the fractions generated at level 0 and at level 1 of the CBT together with the steps to generate them would have been the same.

Now, consider Fig. 4*a*. A careful examination shows that the total number of unique ways of generating fractions for the three step case is 1 (at level-0) + 4 (at level-1) + 16 (at level-2) = 21. Similarly in Fig. 4*b*, the total number of ways is 1 (at level-0) + 6 (at level-1) + 16 (at level-2) = 23. Moreover in Fig. 4*c*, the number is 1 (at level-0) + 6 (at level-1) + 16 (at level-2) = 23. Hence the sumtotal number of fraction generation methods = 21 + 23 + 23 = 67. Obviously, since the number of unique fractions is 17, these 67 methods contain different ways to generate the same fraction.

However, we list the step sequences for generating 17 unique fractions with $D = 18$ in Table 1. A typical mix-split step written as (2:1)-0-18-6 means two droplets having numerator 0 is mixed with one droplet with numerator 18 to generate a new concentration with numerator 6, the denominator is generally clear from the context. Since the derivation of a closed form for the number of unique ways to generate fractions appears to be difficult in general, we present the following lemma instead.

Lemma 3: A lower bound on the number of ways the fractions can be generated is given by

$$\binom{b+w}{w} \times 2^w \times 2^{b+w-1}.$$

Proof: The total number of ways to generate fractions for which all $b + w$ mix-split steps are used is given by

$$\binom{b+w}{w} \times 2^w \times 2^{b+w-1},$$

and they are all unique. □

For our small example, the above result gives a lower bound of $3 \times 4 \times 4 = 48$, which is <67.

Our preprocessing phase constructs a table that contains the best possible sequences for generating a fraction with respect to each of the five optimising criteria stated before. For the small example with $D = 18$, coincidentally for each of the 17 fractions, the optimal sequence turned out to be the same. We report these 17

Table 2 Optimal step sequences for generating 84/144

Steps	Wt. steps	Waste	Input sample	Input buffer	Sequences			
					Step 1	Step 2	Step 3	Step 4
3	1	3	3	2	(1:1)-0-144-72	(2:1)-72-144-96	(1:1)-72-96-84	
4	1	**2**	**2**	2	(1:2)-0-144-96	(1:1)-0-96-48	(1:1)-48-96-72	(1:1)-72-96-84

optimal sequences in Table 1. However, for a mixing example where $b=4$ and $w=2$, then $D=2^4\times3^2=144$. For generating a fraction with numerator 84, we found two different sequences shown in Table 2 being better than all other sequences. The first one optimises the first criterion, that is, the total number of steps and the second one optimises the third and fourth criteria, that is, the number of waste droplets and buffers. However, from the point of second and fifth criteria both the sequences perform equally. In our methodology, we have followed certain convention for tie resolution, if any.

The mix-split step sequence for generating $C_t=5/18$ from buffer (0/18) and sample (18/18) is shown in Fig. 5. The required sequence is taken from 8th, 9th and 10th columns of the 5th row of Table 1.

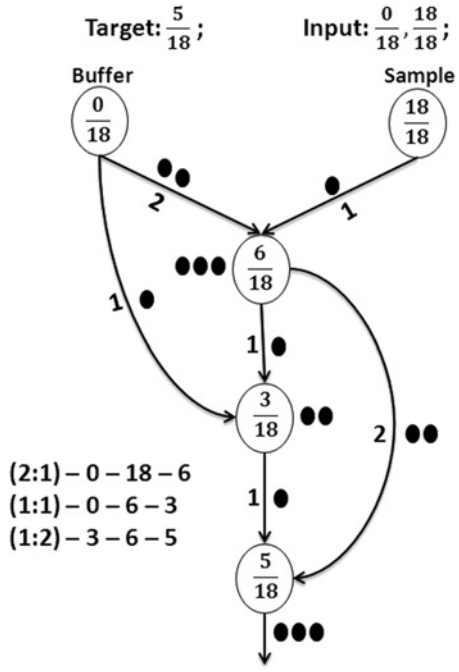

Fig. 5 *Weighted mix-split steps for generating target CF = 5/18 from buffer (0/18) and 100% sample (18/18)*

Let us consider another example with $b=11$ and $w=4$. Hence, denominator $=2^{11}\times3^4=165,888$. Now

$$\binom{15}{2}=1365.$$

We multiply 1365 *BW*-sequences with $2^w=2^4$ weighted arrangements to get $1365\times16=21,840$. Using Lemmas 2 and 3, we respectively get the upper and lower bounds as $21,840\times(2^{15}-1)=715,653,120$ and $21,840\times2^{14}=357,826,560$.

5.2 Generation of optimal step-sequences

We have designed a simulation engine that keeps on generating a new fraction in each step by performing a mix-split step within a particular range. It iteratively tries out all possible ranges of all possible sequences. After generating a new fraction, it calls a procedure that reports the five different parameters such as number of steps, number of weighted steps, number of waste droplets. We keep on updating the look-up table to store the best solution so far obtained.

However, the decision is not taken by the comparison of a single parameter. A hierarchical comparison of a maximum of five parameters is done, in case there are ties. As for example, if we are choosing an optimal sequence for minimising waste droplets, we first compare the number of waste droplets, then in case of a tie, compare the number of weighted steps as they are expensive. In case of another tie, we compare the number of total steps, then in case of tie here also, we compare the fourth parameter and then the fifth. When all possible fraction-compositions are exhausted, the final table that lists the optimal solutions is constructed.

Note that for a particular fraction, the number of possible ways a fraction can be optimally composed is at most five, if they are all different for each parameter. However, such cases rarely arise. As in Table 3, we find that the entry in the first row, 8th column is 17, that is, $D-1$, since for each fraction the same sequence turns out to be the optimal one with respect to all five parameters. However in other rows, the entries are much more than that but still far less than $5\times(D-1)$. Note that since the entries in the 7th column are far smaller than the number of ways tried out we can store the results of our simulation for future use.

The CPU time reported in the last column shows that these simulations are not very expensive to perform off-line. The simulation engine has been performed on a Intel (R) Core (TM)

Table 3 Experimental results for the preprocessing phase

Trials	B	W	T	Denom. D	No. of ways to generate fractions			Time hh:mm:ss
					Upper B.	Lower B.	Optimal	
1	1	2	3	18	84	48	17	00:00:00
2	4	2	6	144	3780	1920	161	00:00:00
3	8	2	10	2304	184,140	92,160	3029	00:00:04
4	7	3	10	3456	982,080	491,520	4879	00:00:37
5	6	4	10	5184	3,437,280	1,720,320	7876	00:03:35
6	9	2	11	4608	450,340	225,280	6249	00:00:11
7	8	3	11	6912	2,702,040	1,351,680	10,093	00:02:00
8	7	4	11	10,368	10,808,160	5,406,720	16,353	00:12:22
9	9	3	12	13,824	7,207,200	3,604,480	20,602	00:05:51
10	8	4	12	20,736	32,432,400	16,220,160	33,833	00:49:53
11	11	2	13	18,432	2,555,592	1,277,952	25,786	00:00:49
12	12	2	14	36,864	5,963,412	2,981,888	52,013	00:04:57

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Table 4 Comparative results of balanced, that is, (1:1) and weighted schemes

$B+W$ steps (target *CFs*)	Total step cost	Avg. step count	% Savings in steps	Avg. sample count	Avg. buffer count	Avg. waste count	Avg. waste/(avg. sample + avg. buffer)	% savings in waste
$10B+0W$ (1023)	$10\times1=10$	9.01	6.22	3.51	3.51	5.02	0.72	13.89
$7B+2W$ (1151)	$7\times1+2\times1.5=10$	8.45		3.8	3.8	4.71	0.62	
$11B+0W$ (2047)	$11\times1=11$	10.01	14.79	3.78	3.78	5.57	0.74	17.57
$7B+3W$ (3455)	$7\times1+3\times1.5=11.5$	8.53		3.39	3.39	4.15	0.61	
$12B+0W$ (4095)	$12\times1=12$	11	22.45	4.06	4.06	6.12	0.75	18.67
$7B+3W$ (3455)	$7\times1+3\times1.5=11.5$	8.53		3.39	3.39	4.15	0.61	
$12B+0W$ (4095)	$12\times1=12$	11	22.55	4.06	4.06	6.12	0.75	18.67
$6B+4W$ (5183)	$6\times1+4\times1.5=12$	8.52		3.54	3.54	4.32	0.61	
$8B+0W$ (255)	$8\times1=8$	7.03	28.73	2.98	2.98	3.96	0.66	16.67
$1B+5W$ (485)	$1+5\times1.5=8.5$	5.01		3.32	3.32	3.63	0.55	
$9B+0W$ (511)	$9\times1=9$	8.02	37.53	3.24	3.24	4.49	0.69	20.29
$1B+5W$ (485)	$1+5\times1.5=8.5$	5.01		3.32	3.32	3.63	0.55	
Avg. % Savings			**22.05**					**17.63**

*i*3–2120 machine with a processor speed of *3.30 GHz* and *1.90 GB* of RAM.

5.3 Comparative study

We have compared the results of our simulation for four different weighted schemes with balanced schemes having comparable denominators, as shown in Table 4. We compared them in terms of total step cost, average step count, % savings in steps, average sample count, average buffer count, average waste count and % savings in waste. Note that the step cost (mixing time) for (1:1) balanced mixing/splitting is considered as 1.0, whereas that for (1:2) weighted mixing/splitting is considered as 1.5, as in the *WD* case three droplets are manipulated in contrast to two droplets for the balanced case. In the first part of 1st row of Table 4, we have used $10B+0W$, that is, $b=10$ and $w=0$ giving $D=2^{10}\times3^0=1024$. To obtain a comparable precision, in the second part of the same row, for $7B+2W$, we have used $b=7$ and $w=2$ generating $D=2^7\times3^2=1152$. We have compared the optimisation parameters for 1023 target *CF*s in balanced and 1151 target *CF*s in *WD* dilution methods and the corresponding plots are shown in Fig. 6. It is observed that the total step-cost for both balanced and *WD* schemes are the same, that is, $10\times1=10$ for balanced and $7\times1+2\times1.5=10$ for weighted. On the basis of average-step-count 9.01 and 8.45 (3rd column of 1st

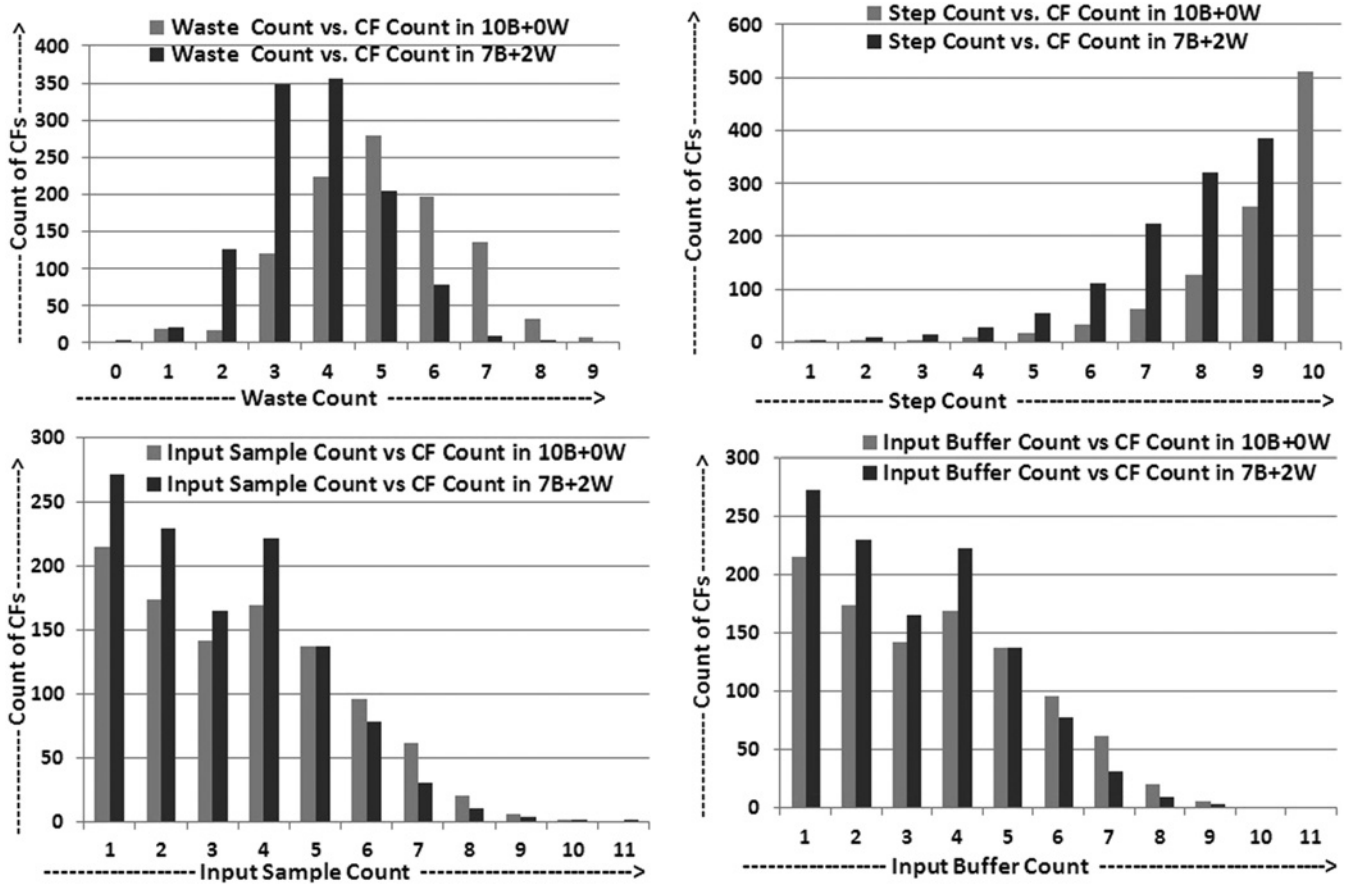

Fig. 6 *Comparison of optimisation Parameters for (7B + 2W) with (10B + 0W)*

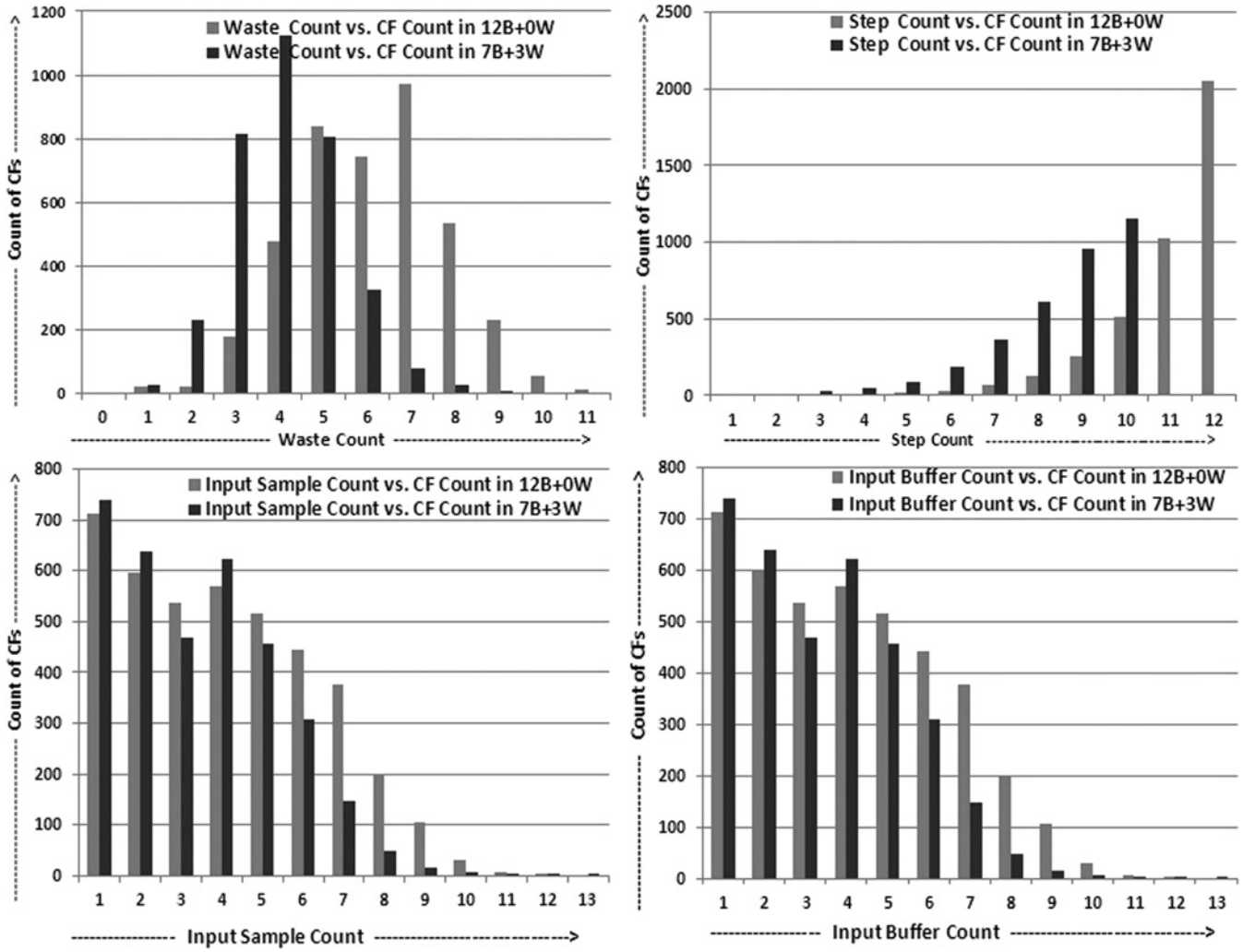

Fig. 7 *Comparison of optimisation Parameters for (7B + 3W) with (12B + 0W)*

row), we compute % savings in steps as $\{(9.01 - 8.45)/9.01\} \times 100 = 6.22$.

We utilise the average values of sample, buffer and waste count for both balanced and weighted models to get $\{5.02/(2 \times 3.51)\} = 0.72$ and $\{4.71/(2 \times 3.8)\} = 0.62$ respectively (8th column of 1st row of Table 4). *WD* reduces the waste significantly compared with the balanced technique giving a % savings of $\{(0.72 - 0.62)/0.72\} \times 100 = 13.89$ (shown in the 9th column of 1st row of Table 4).

Similarly, we have carried out the comparative analysis for ($11B + 0W$ with $7B + 3W$), ($12B + 0W$ with $7B + 3W$), ($12B + 0W$ with $6B + 4W$), ($8B + 0W$ with $1B + 5W$) and ($9B + 0W$ with $1B + 5W$). The results are captured in the Table 4. In the first part of the 3rd row of the same table, we have used $12B + 0W$, that is, $b = 12$ and $w = 0$ creating $D = 2^{12} \times 3^0 = 4096$. In contrast, in the same row, for $7B + 3W$, we can generate $D = 2^7 \times 3^3 = 3456$ with $b = 7$ and $w = 3$. Optimisation parameters for 4095 target *CF*s in balanced and 3455 target *CF*s in *WD* dilution schemes are compared and the corresponding performance plots are shown in Fig. 7. Note that in both Figs. 6 and 7, the bars shown in brown colour are shorter towards the right in each of the plots, that is, for each criterion, the values for *WD* scheme are relatively smaller compared with the balanced scheme on an overall basis.

Note that the number of sequential mix-split steps used in the mixing algorithm determines the accuracy of the target concentration that can be achieved on-chip. It has been observed that for the (1:1) mixing model, 9 or 10 steps provide sufficient accuracy in target concentration (if n denotes the number of (1:1) mix-split steps, the maximum error in target concentration cannot exceed $(1/2^n)$ [14–16]. Thus, in our experiments, we have used at most 12 balanced (1:1) mix-split steps and compared the results with the *WD*-model.

6 Conclusion

We have investigated the problem of diluting a sample using a generalised (weighted) mix-split model. Our analysis shows that by introducing a few (1:2) mix-split steps along with the traditional (1:1) steps in mixing operations, the performance of dilution algorithms can be improved. We also present an architecture of electrode layout that can be used to implement weighted mix-split operations. With certain offline preprocessing, the optimal mix-split steps can be computed *a priori*, and stored as small look-up tables. This table can later be used to generate the required actuation sequence when dilution of a sample is needed in real time. As an open problem, further generalisation of the weighted model may be investigated along with the development of appropriate on-chip sample preparation algorithms that will produce an optimum mix-split sequence.

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