INDIAN INSTITUTE OF ENGINEERING SCIENCE AND TECHNOLOGY, SHIBPUR

Algorithms for Digital Microfluidic Biochips Design and Test

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A report submitted in fulfillment of the requirements for the major project of Bachelor of Technology in Information Technology

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Certificate of Approval

This is to certify that the thesis synopsis entitled **"Algorithms for Digital Microfluidic Biochips Design and Test"** is a record of bona fide work carried out by Mr. Pankaj Kumar Chaurasiya, Mr. Sushanta Soren, Mr. Jyotibrata Sana and Mr. Abhishek Kumar Dubey under my supervision and guidance.

The report has fulfilled the requirements for the completion of a major project of degree of Bachelor of Technology in Information Technology from Indian Institute of Engineering Science and Technology, Shibpur, India.

They have duly completed the required course/research work with sincerity and the work has reached the standard necessary for submission.

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FORWARD

I would like to forward the report entitled Algorithms for Digital Microfluidic Biochips Design and Test Towards the examination committee as a record of bona fide work carried out by Pankaj Kumar Chaurasiya, Sushanta Soren, Jyotibrata Sana and Abhishek Kumar Dubey under my supervision and guidance.

In my opinion, the work for the report is satisfactory and it has reached the standard necessary for the submission in the eight semester of Bachelor of Technology in Information Technology of Indian Institute of Engineering Science and Technology, Shibpur.

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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 have to find an efficient approach to prepare a single sample preparation . Our main purpose is to find a way of mixing two samples(1:1) so that the cost will be minimal and there will be a minimum number of waste droplets.

Introduction

A new field of interdisciplinary research focusing on "lab-on-a-chip (LoC)" is emerging to solve the challenges of healthcare cost for cardiovascular illnesses, cancer, diabetes, the worldwide HIV crisis, and other issues [1]. On a single chip the size of a few square centimeters, a LoC typically conducts one or more biochemical laboratory processes or experiments.

A DMF biochip consists of a 2D array of electrodes, on which discrete droplets of biochemical fluids are manipulated using electrical actuation [2].In many biochemical protocols, solution preparation is a preprocessing step for mixing one or more fluids with a given ratio. Dilution of a biochemical sample/reagent is the special case of mixing where a given fluid is mixed with a buffer solution at a certain ratio corresponding to the desired concentration factor. Dilution is commonly used in biological studies to create a variety of concentrations of the stock solution by mixing it with its diluent on a microfluidic device.

Since some reactants like costly reagents and infant's blood are valuable, their usage should be minimized during dilution.In this project we propose an algorithm for reducing waste droplets and reactant minimization generated during the dilution process on a DMF biochip.[1]

Schematic Diagram

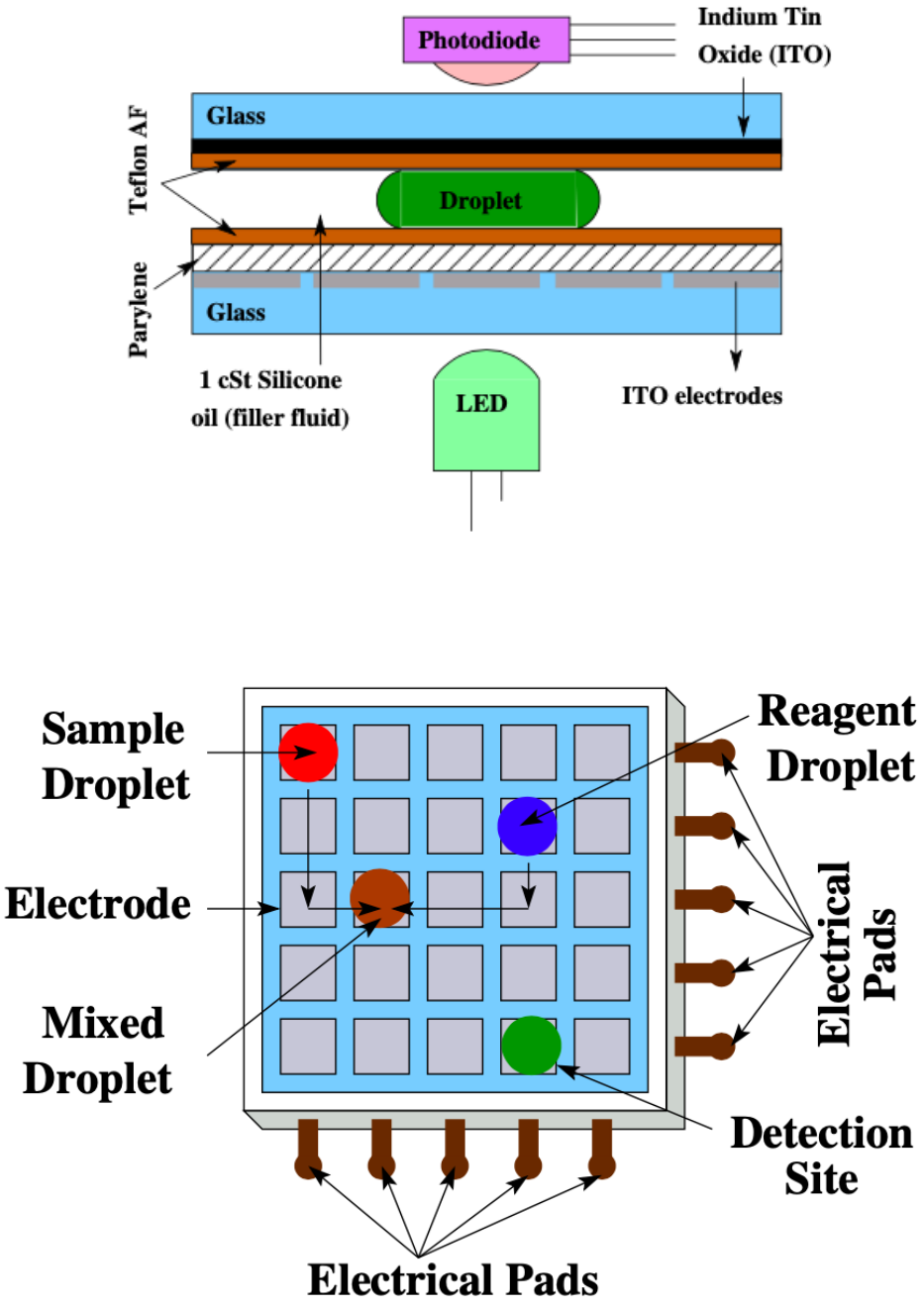

Fig. 1. Schematic of a DMF biochip [1].

Background and Prior Work

The first algorithm designed for dilution control on biochip was based on a binary search to decide the concentrations of intermediate droplets and achieve the target concentration through serial dilution. Recent works addressing the sample preparation problem include BS , DMRW , IDMA , intermediate droplet sharing algorithm (IDSA) [23], and ratioed mixing algorithm (RMA) [24]. Among them, BS, DMRW, and IDMA, address the problem of single-target sample preparation.

Bit-Scanning Method(BS):

The BS approach regards a target concentration as a binary string to guide the entire dilution sequence. A bit '1' in the string indicates that a sample droplet should be dispensed for mixing, and a bit '0' requests for a buffer droplet instead. For example, if Ct =607 1024=0.10010111112, the dilution sequence follows the binary sequence from the least significant bit (LSB) to the most significant bit (MSB) as shown in Figure 2. The length of the string determines the number of dilution operations. An inherent nature of the BS approach is that only one intermediate droplet needs to be kept at all times. Once a dilution operation is completed, one of the two resultant droplets is kept, and the other is discarded. The remaining droplet is then turned into one of the two source droplets for the next dilution operation. According to this nature, BS doesn't need an extra space for storing a large amount of intermediate droplets, and the chip area can thus be reduced. However, it also implies there is no chance for droplet sharing in BS. Hence, the BS method consumes more samples and buffers as well as produces more wastes as compared to other approaches.

Fig. 2. Graph of BS Method[6].

Dilution and Mixing with Reduced Wastage (DMRW) Algorithm:

DMRW is another method for sample preparation and is the first one considering intermediate droplet sharing for waste droplet minimization. DMRW approaches the target concentration based on a binary search strategy[4]. DMRW keeps track of the lower and upper bounds during the entire search process and the two bounds are initially set as 0 and 1 (i.e., buffer and sample). In each iteration, the lower/upper bound is updated with the average of the two if the target is larger/smaller than the average. The process is not terminated until the target is achieved. Since droplet sharing is considered in DMRW, an extra chip space is required for storing intermediate droplets. The dilution sequence through DMRW is illustrated in the given figure, where the number beside an edge indicates the amount of required droplets, and every black node represents a waste droplet. In this case, 6 sample droplets and 4 buffer droplets are required in the entire dilution process. Compared with BS, DMRW generally consumes few sample and buffer droplets, but spends more time for sample preparation.

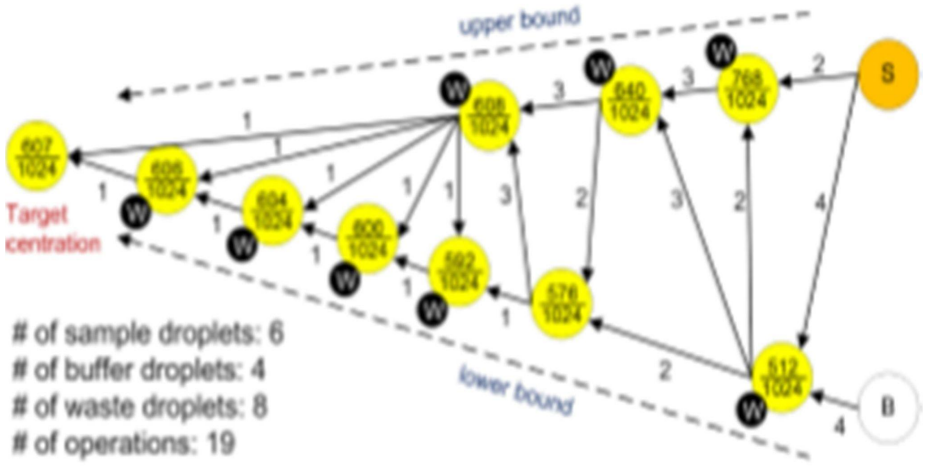

Fig. 3.Graph of DMRW Method[6].

Improved Dilution Mixing Algorithm(IDMA):

IDMA is an improved version of DMRW. For some target concentrations, the mixing graph produced by DMRW is extremely unbalanced. In such cases, more reactants (sample and buffer) are required, and more wastes are produced. IDMA would moderately relax the bound under certain conditions to avoid producing extremely unbalanced graphs[7].It results in a more balanced graph and thus less waste droplets. However, IDMA makes improvements for just a few cases. For most cases, IDMA even consumes more reactants, produces more wastes, and requires more mixing operations. Moreover, IDMA cannot guarantee to always achieve the target concentration.

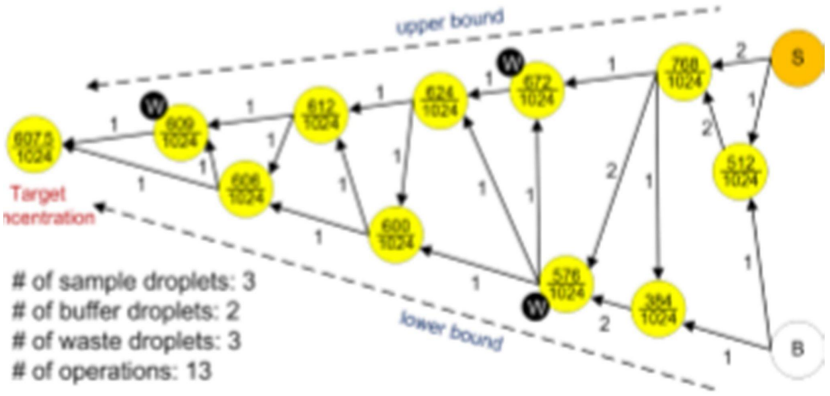

Fig. 4.Graph of IDMA Method[6].

Problem Formulation

The problem of dilution can be stated as follows. Given the supply of a sample or reagent (with 100% conc.) and a neutral buffer solution (0% conc.), determine a sequence of (1 : 1) mix/split steps for obtaining a droplet with a desired target *CF* of the sample. In a more generalized version of the problem, the sample is supplied with one arbitrary *CF*, C_h higher than the target *CF*, and the goal is to determine the mix/split sequence to produce the target sample. For a neutral buffer solution, $C_l = 0$. There are three important issues to be resolved in executing the dilution process: 1) developing an efficient algorithm for determining the mix/split sequence; 2) minimization of the length of the sequence, i.e., actual on-chip dilution time in order to achieve a certain precision of *CF*; 3) reduction of the number of waste droplets for producing a given number of target droplets.

In (1 : 1) mixing model as defined earlier, mixing of two unit-volume droplets of *CFs*, X and Y [denoted as mix(*X*, *Y*)], yields a mixture of $CF = (X+Y)/2$ Thus, to limit the error in target *CF* C_t by $1/2^n$, 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.

Proposed Approach

4.1. Algorithm Overview

During each mixing step two droplets of similar concentration are produced and they are to be disintegrated for further continuation. One droplet will be used for mixing further and another will be added to the waste droplet list. If the reagent droplet is used we assign a cost of 2 units else if a buffer(water) droplet is used we assign a cost of 1 unit else a waste droplet is used and we assign a cost of 0 units. We choose the path with minimum cost. If multiple paths have the same cost then we choose the path with the least number of waste droplets. We choose the path with minimum cost. If multiple paths have the same cost then we choose the path with the least number of waste droplets.

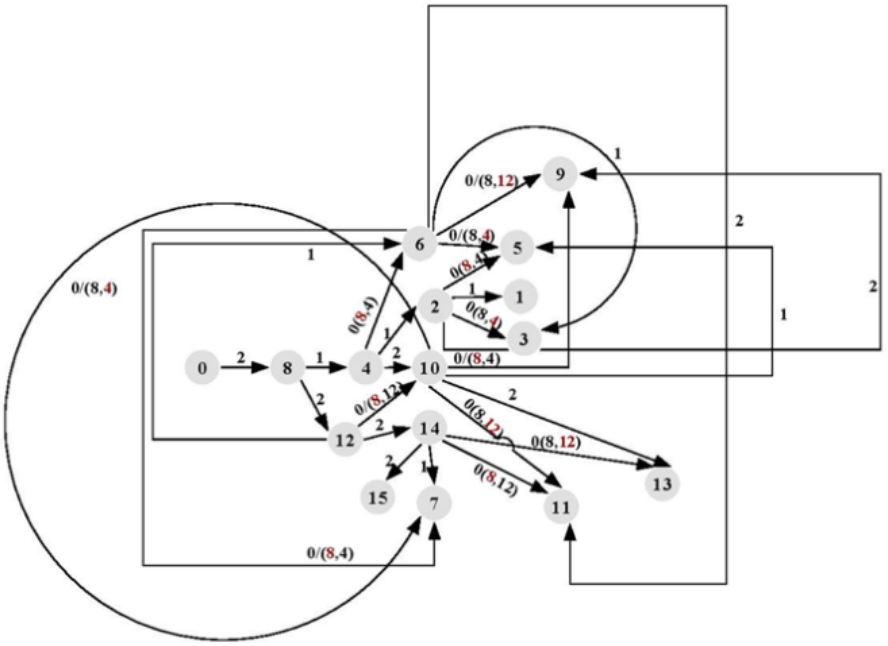

Fig. 3.Graph of Proposed Approach

4.2.Proposed Algorithm

Algorithm 1

1.Let C be the maximum concentration and R be the required concentration and c be the current concentration and Cost be the required cost. We maintain 2 lists. List Path, to store path. List Waste, to store waste droplets.

```
2.    c<-C/2; cost<-2; P<-{0,C/2}; W<-{}
3.    Path(c,cost,P,W)
4.    if (c == R) then
5.        if (cost<Cost)
6.        Path<-P;
7.        Cost=cost;
8.        Waste<-W;
9.    else if (cost == Cost and W<Waste)
10.       Path<-P;
11.       Cost=cost;
12.       Waste<-W;
13.   else
14.       if ( P does not contain (c+C)/2 ) then
15.           cost=cost+2
16.           P<-P+(c+C)/2
17.           W<-W+c
18.           Path(c,cost,P,W)
19.       if (P does not contain(c)/2) then
20.           cost=cost+1
21.           P<-P+(c/2)
22.           W<-W+c
23.   Path(c,cost,P,W)
24.   for every w in W
25.       if(P does not contain(c+w)/2) then
26.           P<-P+(c+w)/2
27.           W<-W+c
28.   Path(c,cost,P,W)
```

Results

Table 1
Savings in the number of droplets for some example CFS using the proposed scheme

Concentration(x/16)	# of reagents used	# of operations	# of waste droplets
1/16	1	4	3
2/16	1	3	2
3/16	1	4	2
4/16	1	2	1
5/16	1	4	1
6/16	1	3	1
7/16	2	4	2
8/16	1	1	0

Conclusion

Sample preparation is an essential process to biochemical reactions. Several previous works have been proposed for waste minimization. However, in our opinion, a reactant (either sample or expensive reagent) can be extremely valuable, and thus its usage should be minimized in the dilution process. In this paper, we present an integrated scheme for reducing the number of waste droplets or mixing steps during the dilution of two samples on a DMF biochip. In our implemented algorithm for single target concentration the time complexity is much lower than other approaches and the number of waste droplets are also very less compared to others.

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