M. Tech. (Computer Science) Dissertation Series

On Some Problems of Scheduling and Dilution Control in Digital Microfluidic Biochips

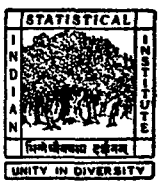
a dissertation submitted in partial fulfilment of the requirements for the M. Tech. (Computer Science) degree of the Indian Statistical Institute

By

Preeti Gupta

under the supervision of

Prof. B. B. Bhattacharya
Advanced Computing and Microelectronics Unit



INDIAN STATISTICAL INSTITUTE
203, Barrackpore Trunk Road
Calcutta-700 035

On Some Problems of Scheduling and Dilution Control

a dissertation submitted in partial fulfilment of the requirements for the M.Tech. (Computer Science) degree of Indian Statistical Institute

By

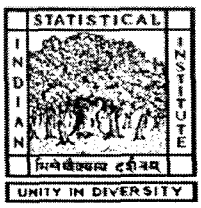
Preeti Gupta

Roll No: CS0724

under the supervision of

Prof. B.B.Bhattacharya

Advanced Computing and Micro electronics Unit



INDIAN STATISTICAL INSTITUTE
203, Barrackpore Trunk Road
Kolkata - 700108

Indian Statistical Institute, Kolkata

CERTIFICATE

This is to certify that the thesis entitled "**On Some Problems of Scheduling and Dilution Control in Digital Microfluidic Biochips**" is submitted by **Preeti Gupta** in the partial fulfillment of the degree of **M. Tech.** in **Computer Science** at **Indian Statistical Institute, Kolkata**. It is fully adequate, in scope and quality as a dissertation for the required degree.

The thesis is a faithful record of bona fide research work carried out by **Preeti Gupta** under my supervision and guidance. It is further certified that no parts of this thesis has been submitted to any other university or institute for the award of any degree or diploma.

Prof. B. B. Bhattacharya
(Supervisor)

P. J. Dasgupta

Countersigned
(External Examiner)
Date: 22/7/09

Abstract

Discrete droplet Digital microfluidic biochips that manipulate discrete droplets face problems similar to those in other VLSI CAD systems, but with new constraints and interrelations. We focus on two such problems: (i) Resource constrained scheduling and (ii) Dilution control of samples. In the first problem we try to minimize intermediate storage as we are performing prefabrication scheduling and therefore, we will obtain an estimate of the number of mixers and storage units keeping number of detectors as a limited resource. We therefore, obtain an estimate of area of the chip to be fabricated along with the valid schedule. Next, we consider the problem of obtaining multiple droplets having different dilution factors, while minimizing number of operations involved. We have developed a method to obtain a single droplet of any arbitrary concentration thereafter, and then used this procedure to obtain multiple droplets of arbitrary concentrations. We have also performed resource constrained scheduling for assays involving droplet dilution.

Acknowledgements

My thanks are due to Professor B·B· Bhattacharya, Advanced Computing and Microelectronics Unit, I·S·I, Kolkatta for supervising my dissertation work. I also thank Sudip Roy for his constant support.

Preeti Gupta

Contents

Chapter 1

Introduction

1.1 Biochips

A biochip is a collection of miniaturized test sites micro arraysarranged on a solid substrate that permits many test sites(micro arrays) arranged on a solid substrate that permits many tests to be performed at the same time in order to achieve higher throughput and speed. Typically biochip area is no longer than a fingernail. Like a computer chip that can perform millions of mathematical operations in one second, biochip can perform thousands of biological reactions, such as decoding of genes in few second.

These chips are essentially miniaturized laboratories that can perform hundreds or thousands of simultaneous biochemical reactions. Biochips enable researchers to quickly screen large number of biological analytes for a variety of purposes, from disease diagonostic to detection of bioterrorism agents.

1.2 Continuous Microfluidic Biochips

Most current microfluidic biochips are based on the principal of continuous flow. They contain permanently etched micropumps, microvalves, microchannel. Their principal is based on continous flow. However continuous microfluidic biochips suffer disadvantages that they are not scalable and they do not offer dynamic reconfigurability. Their fabrication is based on expensive lithographic techniques.

1.3 Digital Microfluidic Biochips

'Digital microfluidics" manipulate liquid as discrete droplet. It's architecture is based on two dimensional microfluidic array of basic cells. They offer dynamic reconfigurability. Group of cells on the 2-D array can be shared among several operations during different time spans. Microcontroller is programmed to access any individual location on a 2-D array. The microcontroller can be reprogrammed latter is a better schedule is found or update itself if a fault is detected. These advantages are not available in continuous flow biochips. The advantages of scalability and reconfigurability make digital microfluidic biochips

a promising platform for massively parallel DNA analysis, automated drug discovery and real time biomolecular detection.

Chapter 2

Intermediate Storage Minimisation in Scheduling

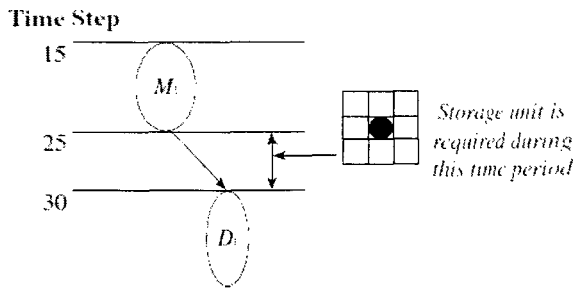
2.1 Scheduling in Biochips

In digital microfluidic biochip we execute bioassays on the chip. We have to determine start and stop times of all operations on the chip which is essential to program the microcontroller so that it can aptly apply the control signal.

2.2 Need for storage

When two operations in the sequencing graph are not scheduled in the consecutive time steps we need storage. It is depicted in the figure below:

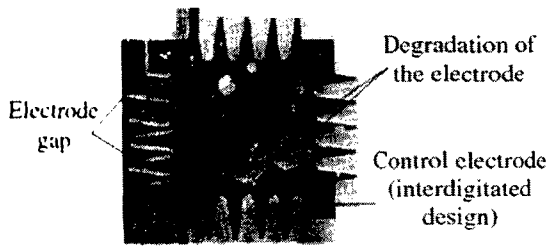
Need for Intermediate Storage



2.3 Minimisation of Storage

The minimization of the assay completion time is essential for environmental monitoring applications where sensors can provide early warning.Real time response is also necessary for surgery and neonatal clinical diagnostics.Long assay durations imply that high actuation voltages need to be maintained on some electrodes, which accelerate electrode degradation and dielectric breakdown,reducing the number of assays that can be performed on a chip during its lifetime.Therefore, assay processing time must be minimized for both disposable and reusable biochip.Electrode degradation is depicted in the figure below:

Top Level View of Faulty Cell : Electrode Degradation



2.4 Problem Formulation

A generic class of micro-droplet-based bioassays protocol can be applied to microfluidic biochips usually consists of the following steps:1)dispensing sample/reagent droplets into the microfluidic array;2)transporting the droplets to some location on the array for assays operations($e \cdot g \cdot$, mixing, dilution or optical detection);3)finally moving the droplets of assay products or wastes out of array. We denote such a generic assay based protocol as P_{assay} and the corresponding sequencing graph as G_p.

We next formulate a scheduling problem whose goal is twofold. First, to determine the start and stop times of all operations, subject to precedence constraints imposed by the sequencing graph. Second, to minimize the storage allocated between two consecutive operations represented by the nodes of the sequencing graph. Resource constraints also needs to be satisfied while bioassay scheduling. In a valid schedule assay operations that share a resource cannot execute concurrently.

In a sequencing graph G_p if there is edge (v_i, v_j) and if v_j cannot be started immediately after the completion of v_i then there is a need for storage allocation. There may be several types of resources for any given bioassay operation. For example, a 2x2 array mixer, a 2x3 array mixer, and a 2x4 array mixer can be used for droplet mixing operation. These mixers differ in their areas as well as mixing times. In such a case a resource selection procedure has to be used.

Based on the above definitions we can formally state our problem as:
Given P_{assay} and it's corresponding sequencing graph G_p and the available resources. Determine a schedule of assays as well as assignment of assay operations to functional resources such that assay completion time is minimized while minimizing storage requirements.

2.5 Solution Approach

We are proposing pre-fabrication synthesis methodology in which resources belonging to the class of reconfigurable are assumed to be available in an unlimited manner. Resources belonging to the class of non-reconfigurable type are not available in abundance. Detectors, reservoirs or dispensing ports belong to the class of non-reconfigurable type. After we have calculated the schedule we can get the idea about the dimension of the two-dimensional array to be fabricated.

We are proposing two pass heuristic. In the first pass we calculate the schedule of all the assay operation with a goal to minimize total time of completion. Second pass is based on backward improvement method whose goal is to avoid or minimize intermediate storage allocated in the first pass.

First we obtain sequencing graph of assay based protocol. Corresponding to each node of a graph we associate $d(v_i)$. For the nodes that corresponds to mixers we use the procedure resource selection. After that we use the pro-

cedure SCHEDULE CONSTRUCT to obtain a valid schedule and to have an estimate the number of mixers required. After that on the output of procedure SCHEDULE CONSTRUCT we run the procedure MODIFY SCHEDULE CONSTRUCT to reduce the requirements of mixers and intermediate storage.

We are not scheduling randomly. We are performing knowledge based scheduling. If any mixing operation have inputs that corresponds to input of sample or reagent. We delay the schedule of such input operations. After the mixing operation is scheduled we schedule input operation one time unit prior to input operation.

2.5.1 Metric

We are defining a metric to estimate the storage requirement as:

$$\text{Metric} = \sum_{i=1}^{n} \text{Tine duration of storage}_i$$
$$\text{where n=number of storage units}$$

We evaluate this metric in the first pass and in the second pass we attempt to reduce this metric value.

2.6 Prefabrication Scheduling Algorithm

ALGORITHM:SCHEDULE CONSTRUCT

Input: Sequencing Graph G(V,E) corresponding to assay based protocol, each node is associated with weight $d(v_i)$and the list $D_1, D_2, ..., D_k$ of distinct detectors available.

Output: Start and stop times of all assay operation, time of completion and the estimate of the requirements of mixers and storage.

Constraints: Detectors and precedence constraints.

1) U1={v_i, $v_i \in$ V and corresponds to the resources of reconfigurable type}.
2) U2={v_j, $v_j \in$ V and corresponds to the input operations}.
3) U3={v_i, $v_i \in$ V and corresponds to the detection}.
4) for each $v_i \in$ U1 do: 5) to 10)
5) If v_i has no parent belonging to U1 do: 6) and 7) else goto 8)
6) $v_i \cdot start \leftarrow 1$
7) $v_i \cdot stop \leftarrow v_i \cdot start + d(v_i)$
8) for v_i having parent $\in$ U1 do 9) and 10)
9) $v_i \cdot start \leftarrow max\{parent_i.stop\}$
10) $v_i \cdot stop \leftarrow v_i \cdot start + d(v_i)$
11) for each $v_i \in$ U2 do: 12) and 13)
12)$v_i.start \leftarrow v_j \cdot start - 1$; where $v_i \rightarrow v_j$
13) $v_i \cdot stop \leftarrow v_i \cdot start + d(v_i)$
14) for each D_i do: 15) to 27)
15) $L_i = \{ v_j : v_j \in$ U3 and $v_j \in$ corresponds to $D_i \}$
16) for each $v_j \in L_i$ do: 17) to 26)
17) t_j=max{ $parent_j \cdot stop$ }
18) sort $v_j \in L_i$ in ascending order based on t_j
19) label the sorted list of L_i as $1, ..., n$ where n is number of operation nodes in L_i.
20) for each i = 1 to n do: 21) to 26)
21) if i = 1 do 22 and 23 else goto 24)
22) $v_j \cdot start \leftarrow t_j$; v_j is the node corresponding to label i
23) $v_j \cdot stop \leftarrow v_j \cdot start + d(v_j)$
24) if $\neq$ 1 do 25) and 26)
25) $v_j \cdot start \leftarrow$ max(t_j, $v_k \cdot stop$),v_k is the node corresponding to label i-1
26) $v_j \cdot stop \leftarrow v_j \cdot start + d(v_j)$

ALGORITHM:MODIFY SCHEDULE CONSTRUCT

INPUT: Output of SCHEDULE CONSTRUCT
OUTPUT: Reduced storage and mixer requirement

1) U={ v_i: v_i corresponds to detection operation}
2) For all v_i do:
3)$v_j \longrightarrow v_i$
4) If there is a storage between v_j and v_i do:
5) Then for all v_k such that
6)t_{min}=min($v_k \cdot start$) and $v_j \longrightarrow v_k$
7) $v_j \cdot stop \longrightarrow t_{min} - 1$
8) $v_j \cdot start \longrightarrow v_j \cdot stop - d(v_j)$
9) j=i
10)Repeat step (3) to (9) till v_i has no parent.

Procedure Resource Selection
1) U={ v_i: v_i corresponds to mixing operation}
2)Generate a random number R between 0 and 1
3) If($R \leq \cdot 3$)
4)Allocate 2×2 mixer
5)If($R \geq \cdot 6$)
6)Allocate 2×3 mixer
7)Else allocate 2×4 mixer.

Procedure:Compute Metric
1) Metric $\leftarrow$ 0
2) for each $v_i \in$ V do:3) and 4)
3) If there exists v_j such that $v_i \rightarrow v_j$ do: 4)
4) Metric $\leftarrow$ Metric + ($v_j \cdot start - v_i \cdot stop$)

2.7 Evaluation Experiments

In this section we present simulation result to evaluate the proposed two pass heuristics.

Application to Multiplexed In-Vitro Diagnostics on Human Physiological Fluids

The in-vitro measurement of glucose and other metabolites, such as lactate, glutamate and pyruvate, in human physiological fluids is of great importance in clinical diagnosis of metabolic disorders. For instance, the change of regular metabolic parameters in the patient's blood can signal organ damage or dysfunction prior to observable microscopic cellular damages or other symptoms. The feasibility of performing a colorimetric enzyme-kinetic glucose assay on a digital microfluidics-based biochip has been successfully demonstrated in experiments [7, 8]. This full-custom biochip consists of a basic microfluidic platform, which moves and mixes droplets containing biomedical samples and reagents, several reservoirs that store and generate the droplets of samples and reagents, and an integrated optical detection system consisting of a LED and a photodiode; see the figure below.

In addition to glucose assays, the detections of other metabolites such as lactate, glutamate and pyruvate in a digital microfluidicsbased biochip have also been demonstrated recently [7, 8]. Using similar enzymatic reactions and modified reagents, these assays as well as the glucose assay can be integrated to form a multiplexed invitro diagnostics on different human physiological fluids, which can be performed concurrently on a microfluidic biochip.

Five examples are used for evaluation. The details are presented in the tables on the next page.

Five Example Experiments(S1:plasma, S2:Serum, S3:Urine, S4:Saliva, Assay1:Glocose assay, Assay2:Lactate assay, Assay3:Pyruvate Assay, Assay4:Glutamate assay)

Example	Description	Node Weights for Mix Operations	Node Weights for Detection Operations
Example1	S1 and S2 are assayed for Assay1 and Assay2	$d(M_1 = 5$ for S1 $d(M_2 = 3$ for S2	$d(D_1 = 5$ for Assay1 $d(D_2 = 4$ Assay2
Example2	S1 and S2 are Assayed for Assay1, Assay2 and Assay3	$d(M_1 = 5$ for S1 $d(M_2 = 3$ for S2 $d(M_3 = 4$ for S3	$d(D_1 = 5$ for Assay1 $d(D_2 = 4$ for Assay2 $d(D_3 = 6$ for Assay3
Example3	S1, S2, S3 are Assayed for Assay1, Assay2 and Assay3	$d(M_1 = 5$ for S1 $d(M_2 = 3$ for S2 $d(M_3 = 4$ for S3	$d(D_1 = 5$ for Assay1 $d(D_2 = 4$ for Assay2 $d(D_3 = 6$ for Assay3
Example4	S1, S2, S3 are Assayed for Assay1, Assay2, Assay3 and Assay4	$d(M_1 = 5$ for S1 $d(M_2 = 3$ for S2 $d(M_3 = 4$ for S3	$d(D_1 = 5$ for Assay1 $d(D_2 = 4$ for Assay2 $d(D_3 = 6$ for Assay3 $d(D_4 = 5$ for Assay4
Example5	S1, S2, S3 and S4 are Assayed for Assay1, Assay2 Assay3 and Assay4	$d(M_1 = 5$ for S1 $d(M_2 = 3$ for S2 $d(M_3 = 4$ for S3 $d(M_4 = 6$ for S4	$d(D_1 = 5$ for Assay1 $d(D_2 = 4$ for Assay2 $d(D_3 = 6$ for Assay3 $d(D_4 = 5$ for Assay4

Mixing time of various types of mixers are listed in a table below:

Mixer Type	Mixing time for plasma samples	Mixing Time for serum Samples	Mixing Time for Urine Samples	Mixing Time for Saliva Samples
2×2-array mixer	7	5	6	8
2×3-array mixer	6	4	5	7
2×4-array mixer	5	3	4	6

2.8 Results

We have shown the results corresponding to example 1, 2 and 3 while the results in the graphical form for the examples 4 and five are not shown explicitly.

2.8.1 Result of Procedure SCHEDULE CONSTRUCT

The time of completion and the mixers required to achieve so by running the SCHEDULE CONSTRUCT are obtained and is in the table below:

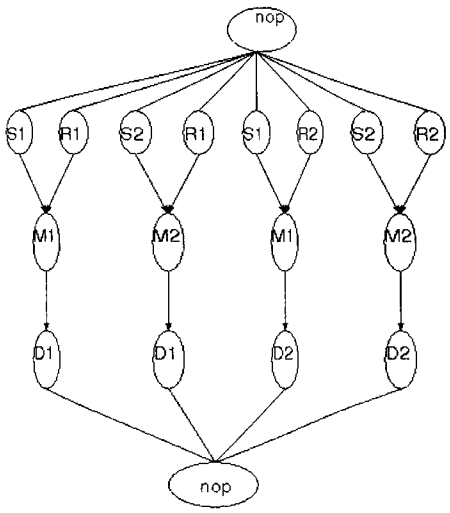
Experiment	Time of Completion	METRIC	Mixers Required
Example1	14	5	4
Example2	17	9	6
Example3	22	35	9
Example4	23	49	12
Example5	27	98	16

2.8.2 Result of Procedure MODIFY SCHEDULE CONSTRUCT

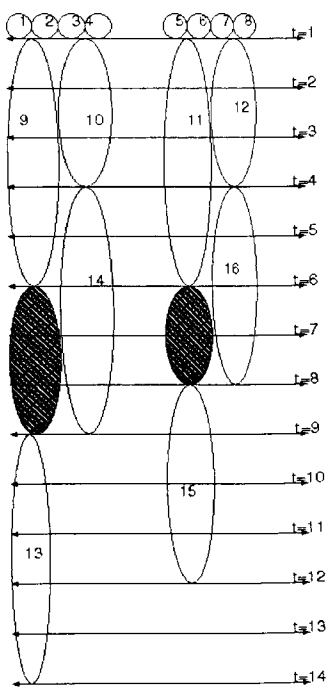
Reduction in the storage metric and the number of mixers required by running the procedure MODIFY SCHEDULE CONSTRUCT is obtained and listed in the table below:

Experiment	Time of Completion	METRIC	Mixers Required
Example1	14	0	3
Example2	17	0	4
Example3	22	0	4
Example4	23	0	4
Example5	27	0	7

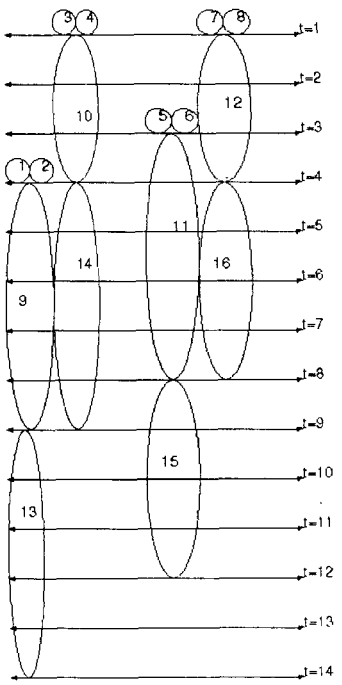
Sequencing Graph Corresponding to example 1



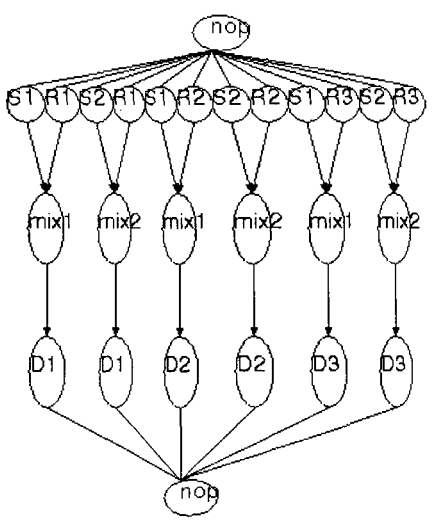
Schedule Obtained Using the procedure SCHEDULE CONSTRUCT for example 1



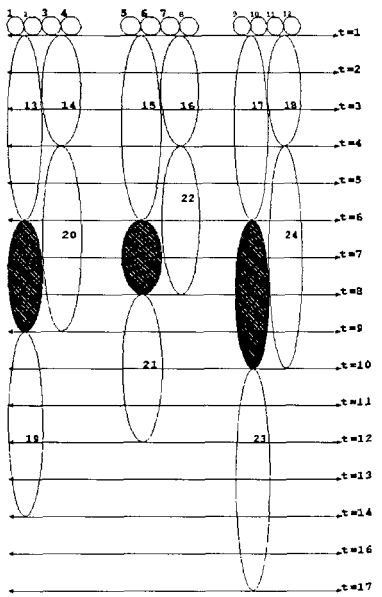
Schedule Obtained Using the procedure MODIFY SCHEDULE CONSTRUCT for example 1



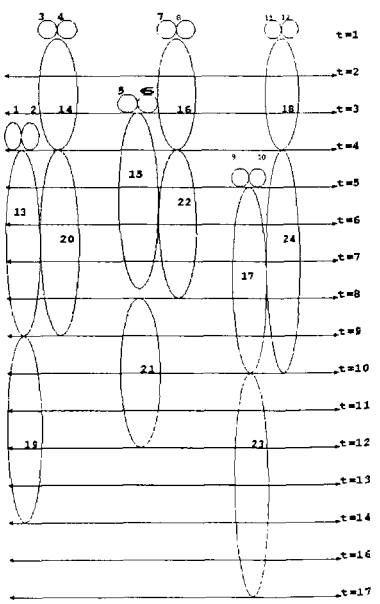
Sequencing Graph Corresponding to example 2



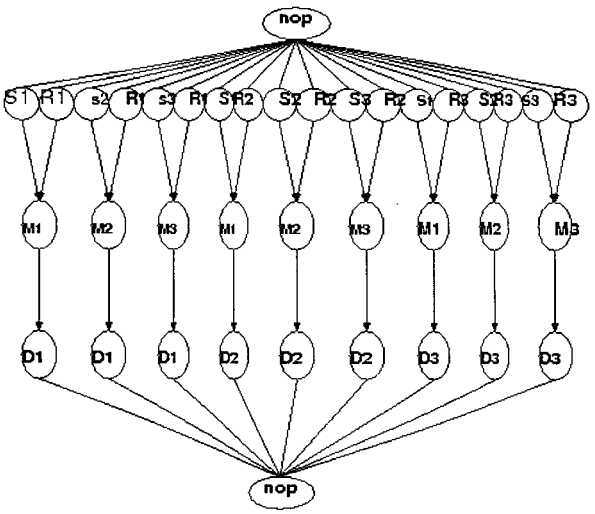
Schedule Obtained Using the procedure SCHEDULE CONSTRUCT for example 1



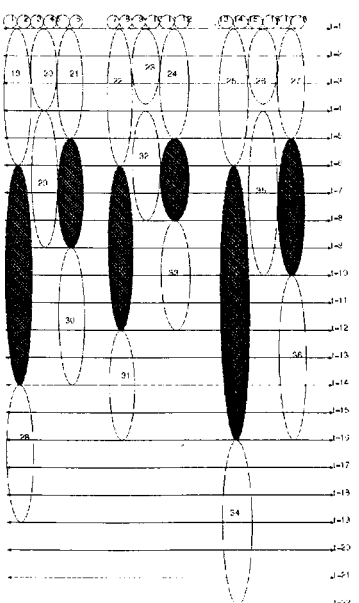
Schedule Obtained Using the procedure MODIFY SCHEDULE CONSTRUCT for example 2



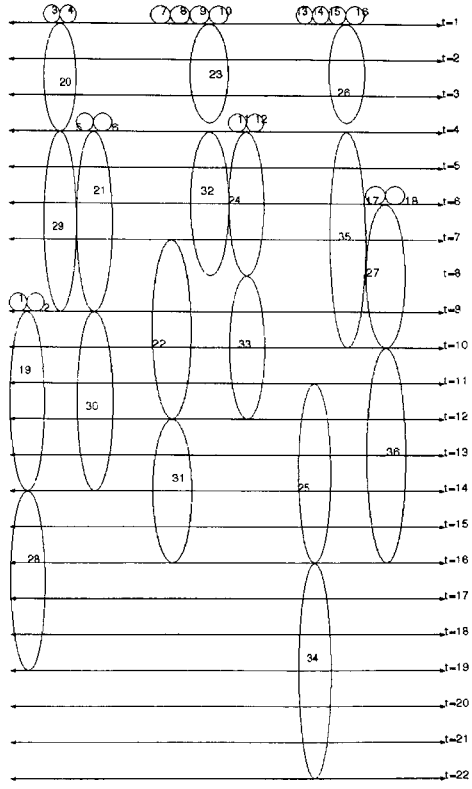
Sequencing Graph Corresponding to example 3



Schedule Obtained Using the procedure SCHEDULE CONSTRUCT for example 3



Schedule Obtained Using the procedure MODIFY SCHEDULE CONSTRUCT for example 3



2.9 Conclusion and Future Work

We have presented a system design methodology that attempts to minimize intermediate storage requirements without exceeding the time of completion. However, because the scheduling problem is *NP* complete we have developed a strategy to minimize storage, and to obtain the number of mixers required based on two pass heuristic. In the first pass we construct optimal schedule with the goal of minimizing time of completion. In the second pass, our strategy is based on backward improvement in which scheduled operations are delayed so that storage requirements are minimized and so that time of completion is not exceeded. A clinical diagnostic procedure, namely multiplexed in-vitro diagnostics on human physiological fluids, has been used to evaluate the proposed methodology. Procedure MODIFY SCHEDULE CONSTRUCT can be applied on the schedule calculated by (M-LS) in [1] and also can be incorporated in GA based approach in [1] to calculate the fitness function.

Chapter 3

Multiple Droplet Dilution

3.1 On-Chip Dilution

Dilution of samples is an important step in almost all bioanalytical systems. The dilution is done as part of the sample preparation process(pre-reaction) and/or during the reaction by controlling sample and reagent volumes. Sample dilution is done primarily for two reasons - to reduce the effect of interfering substances and to increase the linear range of devices.

The ability to digitally manipulate droplets in a electrowetting device enables a new framework for on-chip dilution. The mixing and splitting of two unit droplets to result in an intermediate concentration,is used as a fundamental operation in the proposed scheme. Our approach allows a range of dilution factors to be obtained by using multiple passes of two-droplet mixing and splitting, in various combinations.

3.2 Problem Formulation

Microfluidic biochip involves new type of operation *i·e·* dilution. Buffer droplets are used to dilute the sample to obtain a desired dilution factor(DF). This on-chip dilution is performed using multiple hierarchies of binary mixing/splitting phases,referred to as the interpolating serial dilution method [Fair et al. 2003]. Mixing of sample droplet of concentration C and a unit buffer droplet results in a droplet with twice the unit volume, and concentration C/2. Splitting this large droplet results in two unit-volume droplets of concentration C/2 each. Continuing this step in a recursive manner using diluted droplets as samples,an exponential dilution factor of DF=2^N can be obtained in N steps. Problem becomes difficult if we want a droplet of arbitrary concentration.

3.3 Solution Approach

When we mix droplet of concentration C/m with another droplet of equal volume of concentration C/n we get a droplet of concentration (C/m + C/n)/2. According to this observation each droplet can be referred by a tuple (concentra-

Dilution Factor	Decimal Value	Association
C	1	.5
C/ 2	.5	.25
C/ 4	.25	.125
C/ 8	.125	.0625
C/ 16	.0625	.03125
C/ 32	.03125	.015625
C/ 64	.015625	.007813
C/ 128	.007813	.003906
C/ 256	.003906	.001953
C/ 512	.001953	.000977
C/ 1024	.000977	.000488

Table 3.1: Concentration and Their Association Values in decimal

tion,association) where association is concentration/2. When we decide to mix two droplets having tuple values as (concentration 1,association 1) and (concentration 2,association 2) we get a resultant droplet having concentration association 1+association 2. In the table below all the droplets having $DF = 2^N$where $N = \{1, 2, 3, 4, 5, 6, 7, 8, 9, 10\}$ along with their association value is listed.

3.4 Algorithm for Single Droplet Dilution

Procedure to obtain a droplet having concentration C/k
1)Find N so that $C/2^{N-1} < C/k < C/2^N$
2)$low \leftarrow C/2^{N-1}$
3)$high \leftarrow C/2^N$
4)$mid \leftarrow low \cdot association + high \cdot association$
5)if $low < C/k < mid$ then $high \leftarrow mid$
6)else $low \leftarrow mid$
7)Repeat steps 4,5,6 while $(abs(low - high)) \geq .05)$

3.5 Algorithm for Multiple Droplet Dilution

Procedure to obtain $C/k_1, C/k_2, ..., C/k_n$
1)Sort $C/k_1, C/k_2, ..., C/k_n$ in ascending order
2)for each $i = 1, 2, ..., n$ do
3)if i=1 do
4)Find the mix/split cycles for C/k_i
5)if i$\neq$1 do
6)Find the mix/split cycles for C/k_i
7)Find the wasted droplets in C/k_j ; $j = 1, .., i - 1$
8)Find the used droplets in C/k_i
9)Reduce the mix/split cycles of C/k_i using droplet in 6)

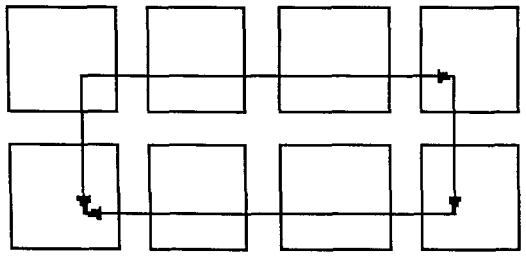
3.6 Simulation

We are conducting eight experiments. In the first five experiments we are using single droplet dilution algorithm, While later three experiments uses multiple droplet dilution algorithm. We are scheduling the corresponding assays on two different chips. One is having two mixers and two storage units while another is having three mixers and five storage units. sample and buffer dispensing operations take 7 seconds. Dilution(mix/split) is performed on a 2x4 array dilutor which takes five seconds.

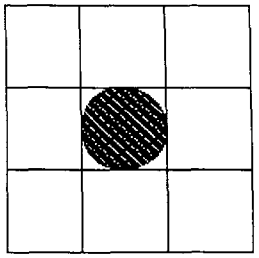
Functional resources for synthesis are listed in table below:

Operation	Resources	Time(s)
Dispensing:sample	On-chip reservoir/dispensing port	7
Dispensing:buffer	On-chip reservoir/dispensing port	7
Dilution	2x4 array dilutor	3

2x4 array dilutor is shown in figure below:

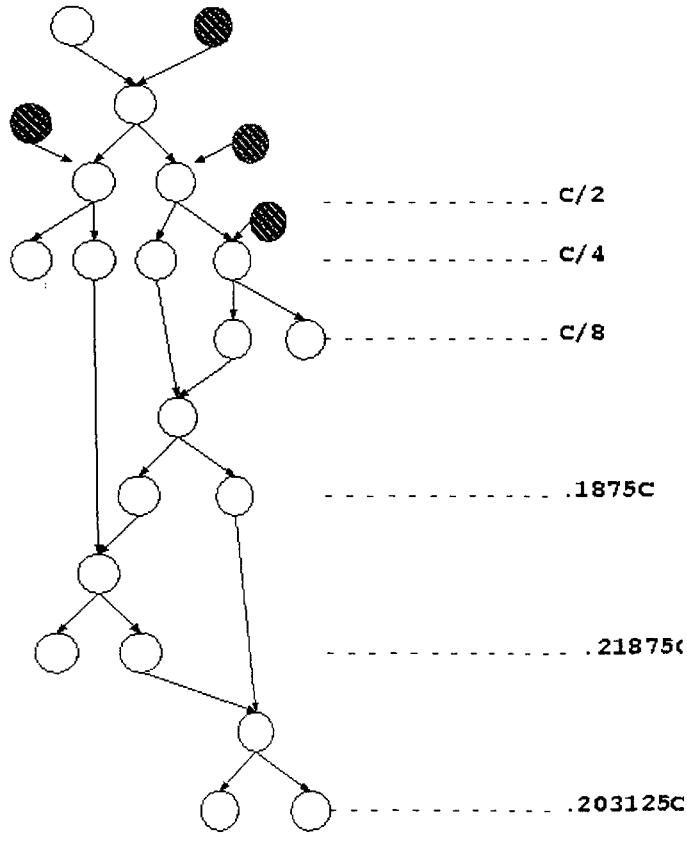


Storage unit is shown in figure below:



3.6.2 Experiment 2

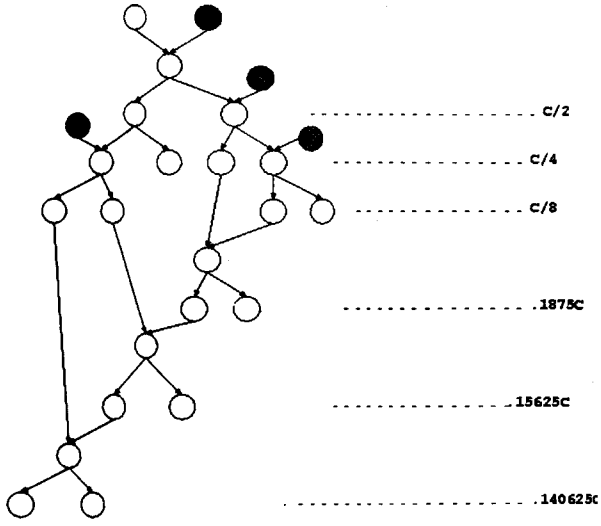
Dilution Factor is C/5 with Error Tolerance ·05
Mixing of ·125C and ·25C gives ·1875C
Mixing of ·25C and ·1875C gives ·21875C
Mixing of ·21875C and ·1875C gives ·203125C
Number of wasted droplets is 4.
Length of the longest path is 8



It requires seven mix/split cycles and four buffer dispensing operations.

3.6.3 Experiment 3

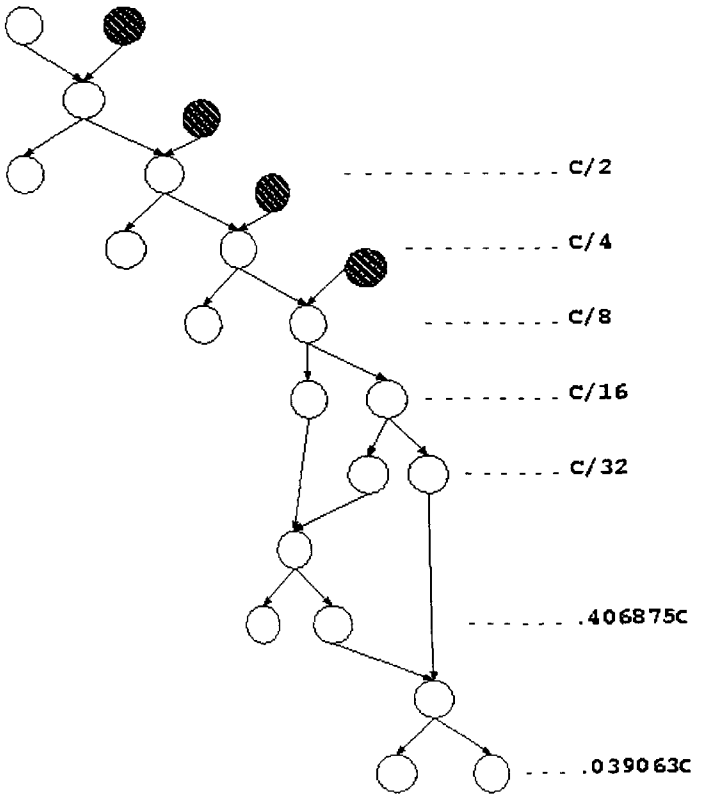
Dilution Factor is C/7 with Error Tolerance ·05
Mixing of ·125C and ·25C gives ·1875C
Mixing of ·1875C and ·125C gives ·15625C
Mixing of ·15625C and ·125C gives ·140625C
Number of wasted droplets is 5
Length of the longest path is 8



It requires eight mix/split cycles and four buffer dispensing operations.

3.6.4 Experiment 4

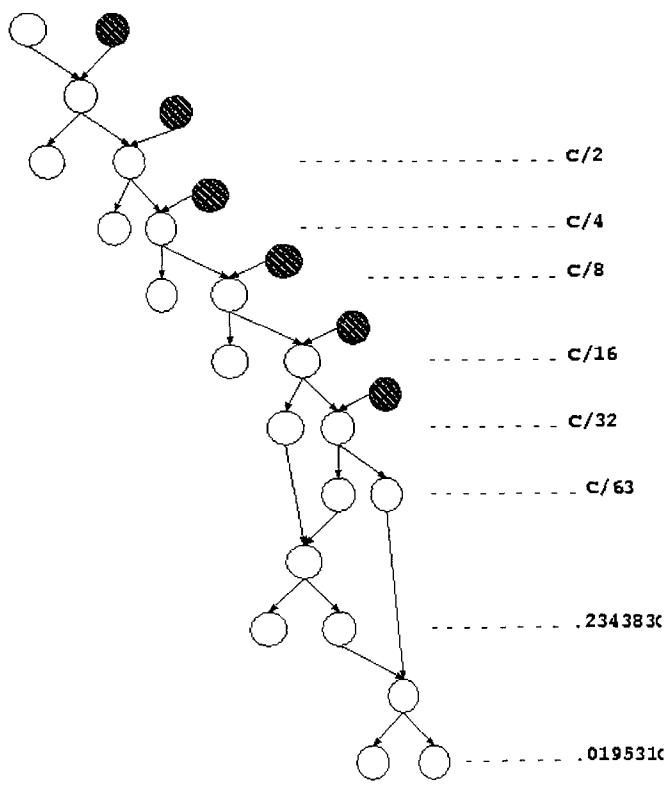
Dilution Factor is C/31 with Error Tolerance ·05
Mixing of ·03125C and ·0625C gives ·046875C
Mixing of ·046875C and ·03125C gives ·039063C
Number of wasted droplets is 5
Length of the longest path is 9



It requires seven mix/split cycle and four buffer dispensing operations.

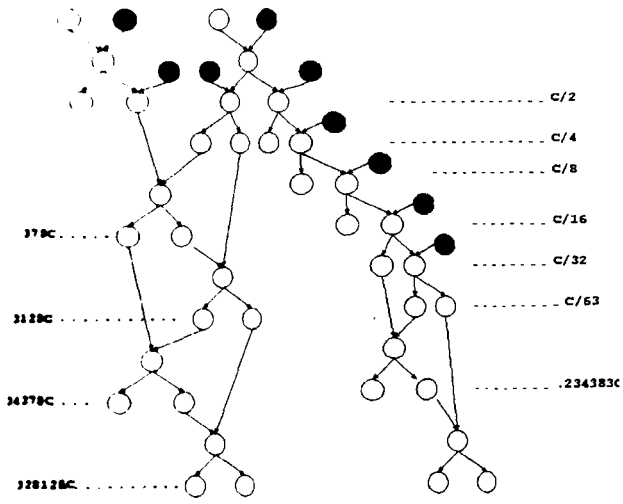
3.6.5 Experiment 5

Dilution Factor is C/63 with Error Tolerance ·05
Mixing of ·015625C and ·03125C gives ·023438C
Mixing of ·023438C and ·015625C gives ·019531C
Number of wasted droplets is 6
Length of the longest path is 11



3.6.6 Experiment 6

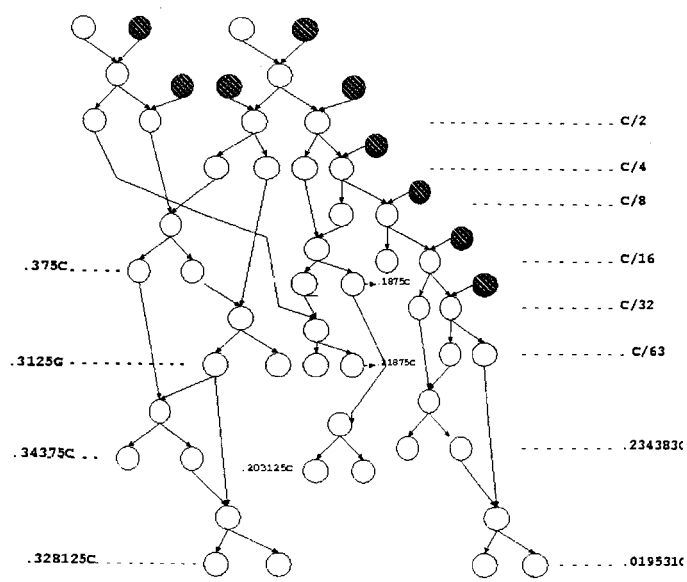
Using multiple droplet dilution algorithm we are obtaining C/3 and C/63 simultaneously.



It requires fourteen mix/split cycles and eight buffer input operations. Number of wasted droplets is 8.

3.6.7 Experiment 7

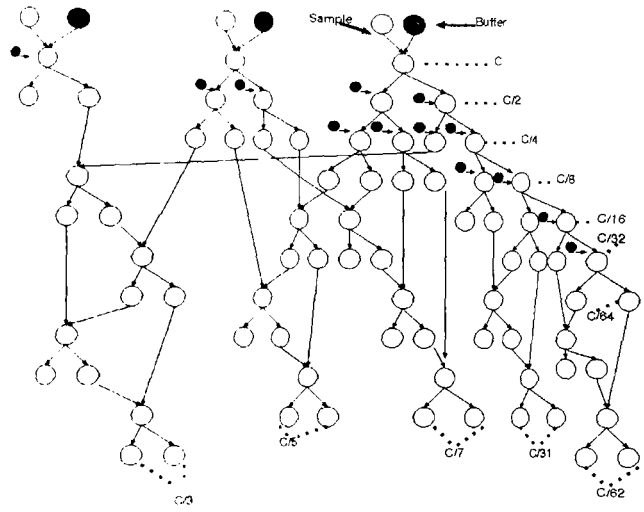
Using multiple droplet dilution algorithm we are obtaining C/3,C/5 and C/63 simultaneously.



It requires seventeen mix/split cycles and nine buffer input operations. While the experiments if performed individually requires total of twenty mix/split cycles and ten buffer input operations. Number of wasted droplets is 9.

3.6.8 Experiment 8

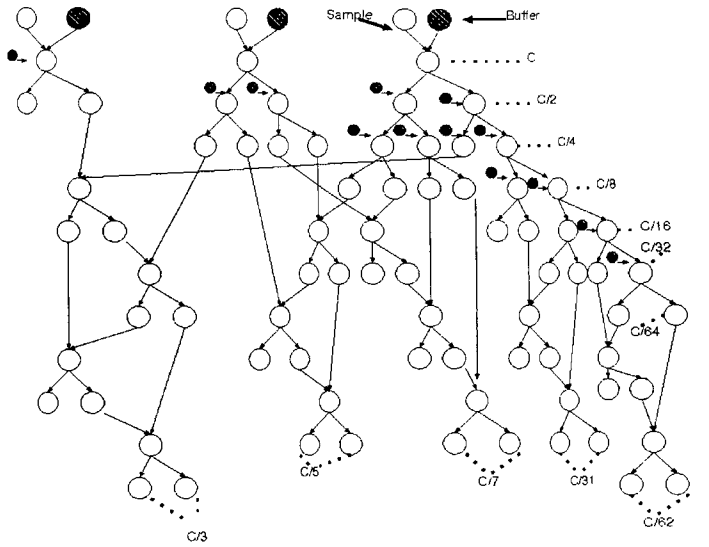
Using multiple droplet dilution algorithm we are obtaining C/3,C/5,C/7,C/31 and C/63 simultaneously. Number of wasted droplets is 12.



It requires Twenty nine mix/split cycles and sixteen buffer input operations and if all the five experiments are performed individually requires thirty five mix/split cycles and eighteen buffer input operations.

3.6.8 Experiment 8

Using multiple droplet dilution algorithm we are obtaining C/3,C/5,C/7,C/31 and C/63 simultaneously. Number of wasted droplets is 12.



It requires Twenty nine mix/split cycles and sixteen buffer input operations and if all the five experiments are performed individually requires thirty five mix/split cycles and eighteen buffer input operations.

3.7 Results

We have used single as well as multiple droplet dilution algorithm and recorded mix/split cycles and number of sample and buffer dispensing operations. Details are listed in table.

Experiment	Mix/Split cycles	Sample Dispensing Operations	Buffer Dispensing Operations
Experiment 1	6	1	2
Experiment 2	7	1	4
Experiment 3	8	1	4
Experiment 4	7	1	4
Experiment 5	7	1	4
Experiment 6	14	2	8
Experiment 7	17	2	10
Experiment 8	29	3	18

We have obtained schedule of assays in the experiments conducted on two different biochips. Completion 1 gives the total time of completion on a chip having two mixers and two storage and completion 2 gives the time of completion on a chip having 3 mixers and five storage units. Results are listed in table below:

Experiment	Completion 1 (2 mixers and 2 storage)	Completion 2 (3 mixers and five storage)
Experiments	37 seconds	37 seconds
Experiment 2	32 seconds	32 seconds
Experiment 3	37 seconds	37 seconds
Experiment 4	42 seconds	42 seconds
Experiment 5	42 seconds	42 seconds
Experiment 6	47 seconds	47 seconds
Experiment 7	67 seconds	52 seconds
Experiment 8	N/A	67 seconds

3.8 Conclusion and Future Work

We have developed an algorithm to obtain a droplet of any arbitrary concentration. We have conducted five experiments to evaluate the proposed method. Later we have conducted three experiments to observe reduction in mix/split cycles if all the diluted droplet of specified concentrations are to be obtained simultaneously.Given the resource constraints we have calculated the total time of completion of all the assays presented in all the experiments. An automated tool has to be developed which would optimize the mix/split cycles if more than one droplet of different concentrations are to be obtained as output.

Bibliography

1. Fei Su and Krishnendu Chakrabarty. High level Synthesis of Digital Microfluidic biochips.*ACM Jornal an Emerging Technologies in Computing Systems*, Vol 3, No. 4,Article 16.

2. F. Su and K. Chakrabarty, "Architectural-Level Synthesis of Digital Microfluidics-Based Biochips", *Proc. IEEE International Conference on CAD*, pp. 223-228, 2004.

3. M.G. Pollack, R.B. Fair, and A.D. Shenderov,"Electrowetting-based actuation of liquid droplets for microfluidic applications", *Applied Physics Letters*, vol. 77,pp. 1725-1726, 2000.

4. F. Su and K. Chakrabarty, "Defect tolerance based on graceful degradation and dynamic reconfiguration for digital microfluidic biochips".*IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems*, vol. 25, pp. 2944-2953, December 2006.

5. Bean J·C 1994. Genetics and random keys for sequencing and optimization.*ORSA J· Comput· 6,154-160*

6. H. Ren and R. B. Fair, "Micro/Nano Liter Droplet Formation and Dispensing by Capacitance Metering and Electorwetting Actuation", *Technical Digest IEEE-NANO*, pp. 36-38, 2002

7. V. Srinivasan, V. K. Pamula, M. G. Pollack and R. B. Fair, "A digital microfluidic biosensor for multianalyte detection". *Proc. IEEE Conf MEMS*, pp. 327-330,2003

8. V. Srinivasan. V. K, Pamula. M. G. Pollack and R. B. Fair. "Clinical diagnostics on human whole blood, plasma, serum, urine, saliva, sweat, and team on a digital microfluidic platform", *Proc. pTAS*, pp.1287-1290,2003.