

DESIGN OF TEST SIMULATOR FOR MICROFLUIDIC BIOCHIPS

BY

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UNDER THE GUIDANCE OF

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**TERM PAPER REPORT
SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS**

**FOR THE DEGREE OF
Bachelor of Engineering (INFORMATION TECHNOLOGY)**

AT
DEPARTMENT OF INFORMATION TECHNOLOGY
BENGAL ENGINEERING AND SCIENCE UNIVERSITY, SHIBPUR
HOWRAH-711103, INDIA
April-2012

BENGAL ENGINEERING AND SCIENCE UNIVERSITY, SHIBPUR
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FORWARD

I/We hereby forward the project entitled "**DESIGN OF TEST SIMULATOR FOR MICROFLUIDIC BIOCHIP**" presented by **Soumen Basu, B.E. 8th Semester, 2012** under my guidance in partial fulfillment of the requirements for the Bachelor Degree in Information Technology of this University.

A handwritten signature in blue ink, appearing to read "Hafizur Rahaman".

Prof. Hafizur Rahaman
Dept of Information Technology
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And

A handwritten signature in blue ink, appearing to read "Pranab Roy".

Prof. Pranab Roy
School of VLSI Technology
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Dated:
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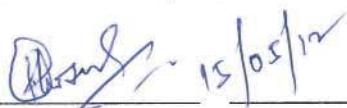
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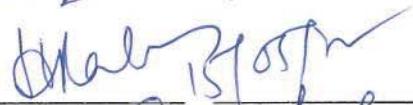


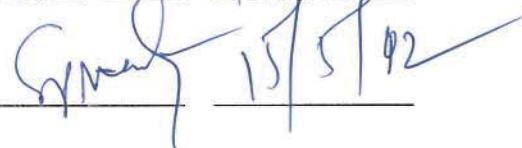
APPROVAL

The project is hereby approved as a creditable study in the area of Information Technology carried out and presented satisfactorily by Soumen Basu to warrant its acceptance as a prerequisite for the award of the Bachelor Degree in Information Technology from Bengal Engineering and Science University, Shibpur. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein, but approve the TERM PAPER only for the purpose for which it is submitted.

Board of Term Paper Examiners:


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Abstract

Microfluidics-based biochips offer a promising platform for massively parallel DNA analysis, automated drug discovery, and real-time bimolecular recognition. The first part of this paper introduces readers to digital microfluidics technology. The next part is focused on test issues. It is important to ensure high reliability and availability of biochips as they are increasingly deployed for safety-critical applications. The paper includes a brief discussion of fault models, concurrent test issues. The main focus of this project is based on different testing namely structural, functional and customized routing test and design of necessary algorithm for optimizing the performance of such testing and hence contribute to the design of the test simulator.

Introduction

Microfluidics-based biochips for biochemical analysis are receiving much attention nowadays. These composite microsystems, also known as lab-on-a-chip or bio-MEMS, offer several advantages over conventional laboratory procedures. They automate highly repetitive laboratory tasks by replacing cumbersome equipment with miniaturized and integrated systems, and they enable the handling of small amounts, e.g., Nano liters, of fluids. Thus they are able to provide ultra-sensitive detection at significantly lower costs per assay than traditional methods, and in a significantly smaller amount of laboratory space. Most microfluidic biochips of today contain permanently etched micro pumps, micro valves, and micro channels, and their operation is based on the principle of continuous fluid flow . A promising alternative is to manipulate liquids as discrete droplets. Following the analogy of microelectronics, this novel approach is referred to as “digital microfluidics”. Each droplet can be controlled independently and each cell in the array has the same structure. Therefore, in contrast to continuous-flow systems, droplet-based microfluidics offers reconfigurability as well as a scalable system architecture. These advantages make digital microfluidics-based biochips a promising platform for massively parallel DNA analysis, automated drug discovery, and real-time bio molecular detection.

Related Studies

- **Digital Microfluidics-Based Biochips:** Microfluidic biochips can be based on manipulation of Nano liter drops using the principle of electrowetting. Electrowetting refers to the modulation of the interfacial tension between a conductive fluid and a solid electrode by applying an electric field between them. The basic cell of a digital microfluidics-based biochip consists of two plates, and the filler medium, i.e., the silicone oil, sandwiched between the plates. The droplets travel inside the filler medium. The bottom plate contains a patterned array of individually controllable electrodes, and the top plate is coated with a continuous ground electrode. By varying the electrical potential along a linear array of electrodes, droplets can be moved along this line of electrodes. The velocity of the droplet can be controlled by adjusting the control voltage (0~90V), and droplets can be moved at speeds of up to 20 cm/s. Based on this principle, microfluidic droplets can be moved freely to any location of a two-dimensional array without the need for micropumps and microvalves.

There are four basic operations that can be performed by microfluidic biochips and their corresponding simulations can be made. The operations are as follows

1. Sample introduction (*Dispense*).
2. Sample movement (*Transport*).
3. Preservation (*Temporary Store*).
4. Mixing different samples (*Mix*).

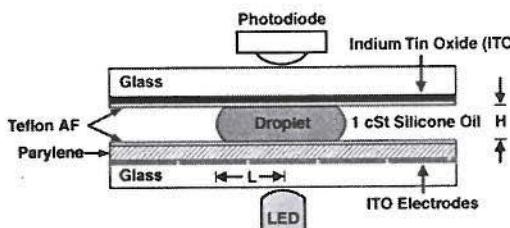


Fig 1: Diagram of the structure

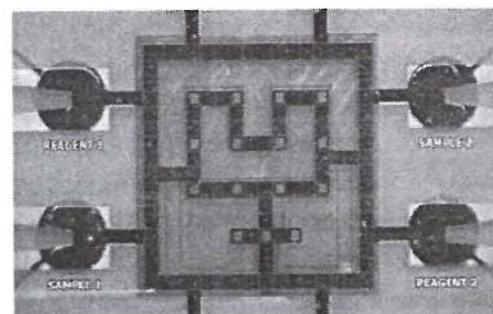


Fig 2: A real microfluidic biochip

Generic Fault Models in Micro fluidic Biochips

Faults in digital microfluidic systems can be classified as being either catastrophic or parametric. Catastrophic (hard) faults lead to a complete malfunction of the system, while parametric (soft) faults cause a deviation in the system performance. A parametric fault is detectable only if this deviation exceeds the tolerance in system performance. **Catastrophic faults** may be caused by the following physical defects:

- **Dielectric breakdown:** The breakdown of the dielectric at high voltage levels creates a short between the droplet and the electrode. As a result, no charge can be stored in the interface. As the electrowetting mechanism depends on the amount of energy stored in the capacitor formed by the electrode and the droplet, dielectric breakdown inhibits fluid motion.
- **Short between the adjacent electrodes:** As a result of a short circuit between two adjacent electrodes, these electrodes effectively form one longer electrode. Thus, the droplet residing on this electrode is no longer large enough to overlap with the adjacent electrodes, inhibiting its actuation.
- **Degradation of the electrode:** This degradation effect is unpredictable and may become catastrophic during the operation of the system. A consequence of electrode degradation is that droplets often fragment and their motion is prevented because of the unwanted variation of surface tension forces along their flow path.
- **Open in the metal connection between the electrode and the control source:** This defect results in a failure of charging electrode while trying to drive the droplet.

Physical defects that cause parametric faults include the following:

- **Geometrical parameter deviation:** The deviation in insulator thickness, electrode length, and height between parallel plates may exceed their tolerance value.
- **Insulator degradation:** This “wear-and-tear” defect may become apparent gradually during operation. If left undetected, it may eventually cause electrode degradation.
- **Particle contamination:** During in-field operation of a microfluidic system, the droplet or the filer fluid may be contaminated by a particle, such as a dust particle or a foreign fluid droplet. Typically such particles are then attached to the surface of the insulator of a cell and affect the motion of the droplet.
- **Change in viscosity of droplet and filler medium.** These deviations can occur during the operation due to an unexpected biochemical reaction, or a defect in the control system causing unwanted temperature variation.

Generic Testing in Micro fluidic Biochips

A. Dispensing Test

The dispensing test targets the malfunctioning of the dispensing operation. Fig. 2 provides a comparison between normal dispensing and an example of dispensing failure. As the dispensed droplet in a malfunctioning scenario cannot be detached from the droplet in the reservoir. Therefore, when we move the dispensed droplet away from the reservoir, an additional droplet from the reservoir is extracted and moved as well. In this case, the dispensed “droplet” can be several times larger than the normal size, which may result in the catastrophic failure of a volume-sensitive bioassay.

B. Routing Test and Capacitive Sensing Test

Routing test focuses on evaluating a single electrode’s ability to transport droplets. This procedure is similar to that proposed earlier for structural test . In the structural test, a test droplet is dispensed and routed to cross the target electrode from two orthogonal directions (i.e., along the row and the column directions). The routing problem can be solved by mapping the array to an undirected graph and applying the Eulerpath-based method. On the other hand, a test droplet must be routed along all four directions relative to the target electrode. We can solve the route planning problem in this case by mapping the target array to a directed graph, which can be easily derived by replacing every edge in the undirected graph with two directed edges in the opposite directions. The Euler-path-based method is then applied to the directed graph to derive a test plan for the routing test.

C. Mixing and Splitting Test

Next, we discuss the functional testing of two widely used microfluidic modules—mixers and splitters. In a digital microfluidic biochip, two droplets are mixed within a cluster of electrodes, referred to as the mixer. Even though mixer designs and configurations vary considerably the underlining mixing mechanisms remain the same for all designs and configurations. Two droplets are merged at one electrode and routed to move about some pivots in the mixer, thus, a mixing functional test is equivalent to the testing of the merging and routing operations within the target cell cluster. Recall that the droplet routing test has been addressed therefore; a mixing test can be reduced to a droplet merging test, which checks a series of three adjacent electrodes to determine whether two droplets can be merged on them. For a microfluidic array, a simple test method carries out droplet merging on every group of three adjacent electrodes.

Proposed Structural Faults and Solution Models

In cases of cross referencing biochips as the electrodes are activated along the peripheral row and columns – majority of fault occurrence may take place along the peripheral pins – as well as occurrence of open Bus faults for a given row or column may be imminent. Based on these issues we have identified certain types of structural faults that may occur frequently in cross referencing Biochips and defined their cause and effects respectively.

The various faults that can occur in a cross referencing biochip are

1. **Stuck at Faults**
2. **Open Bus faults**
3. **Electrode Short**
4. **Misalignment of buses**

The proposed solution models are as follows:

PERIPHERAL SCAN METHOD

This method is specifically used to detect stuck at faults and open bus faults in a given 2D array of a cross referencing Biochips.

1. Two test droplets placed at two end locations (1,1) and (m,n) of a 2D array of m X n dimension being dispensed from a single source.
2. The droplet D1 at (1,1) is transported along row 1 to the given sink location starting at time t = 0.
3. The droplet D2 at (m,n) is transported along column n in the upward direction to the same sink starting at time t=3; The cause of delay of the second droplet D2 is to avoid unwanted mixing before reaching to sink.
4. If a test droplet reaches to visit the electrode connected to the target capacitive sensing circuit at the sink , a positive pulse is expected at the output of the sensing circuit.
5. In such cases if no defect occurs in either of the row electrodes or column electrodes two output pulses will be available at the sensing circuit. (We assume no unwarranted delay in actuation for the droplet occurs).
6. If any or both of the droplets fail to reach the sink – the corresponding pulse will be missing – indicating stuck at occurs at the corresponding row or column.
7. Total number of cells used in the process is calculated as m+n ;
Number of sources used = 1; Number of sinks used = 1;Number of test droplets used = 2;
total time required = m+n+3.

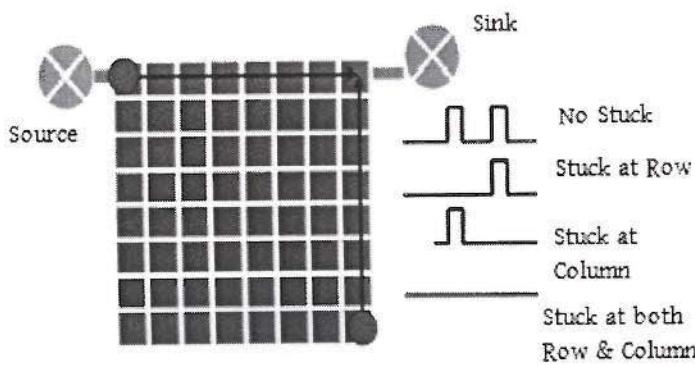


Fig 3: Peripheral Scan Method

PARALLEL SCAN METHOD

1. In this method the start locations are at the array boundary. Multiple test droplets are used for testing .Each start location is considered as pseudo source and each corresponding target location are considered as pseudo sink.
2. Minimum possible spacing is necessary between test droplets i.e of one electrode in order to avoid contamination.
3. This implies that only half of the columns or rows can be tested concurrently in one parallel scan iteration. Hence minimum of two iterations are necessary to complete the parallel scan test along row or column. While carrying out the peripheral scan tests as stated earlier to identify stuck at or open bus fault – if such fault is identified along row or column –parallel column or row tests are carried out accordingly in order to locate the single fault.

1. Parallel column scan test – Two iterations of parallel scan along the columns of the array is carried out .Test covers every single cells and all the edges of each column. Maximum number of droplets required = number of columns/2. Time of completion = $2 \times$ number of rows + $n \times (n-1)/2$. The last term counts for the total time necessary to move each droplet from the pseudo sink to the sink with capacitive sensor. If stuck at fault at Row is already detected – droplets from specific columns fail to reach the sink (identified by the missing pulse in the sensor) – are marked and the corresponding electrodes at those specific columns are diagnosed as faulty. In case of open bus fault (already detected by peripheral scan method) - droplets for the specific column that failed to reach the sink are identified and the corresponding column Bus is identified as open.

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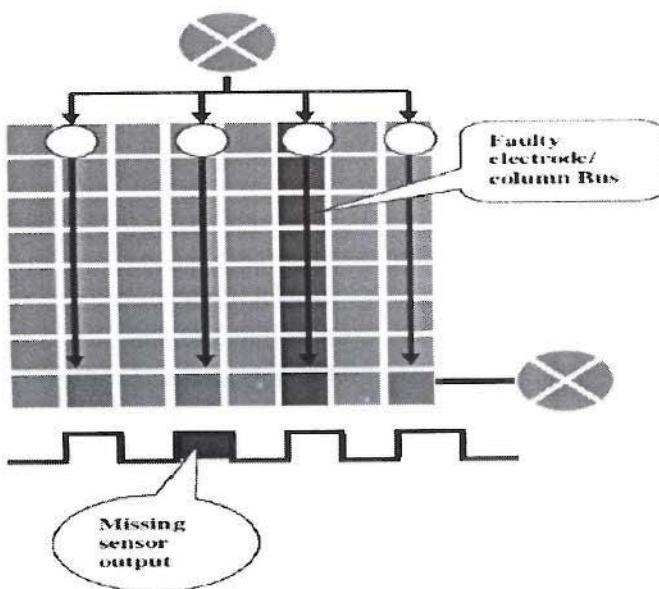


Fig 4 : Parallel Column Scan

2. Parallel Row scan test - Two iterations of parallel scans along the Rows of the array is carried out .The test covers every single cells and all the edges of each Row. Maximum number of droplets required = number of Rows/2. Time of completion = $2 \times$ number of columns + $n \times (n-1)/2$. The last term counts for the total time necessary to move each droplet from the pseudo sink to the sink with capacitive sensor.

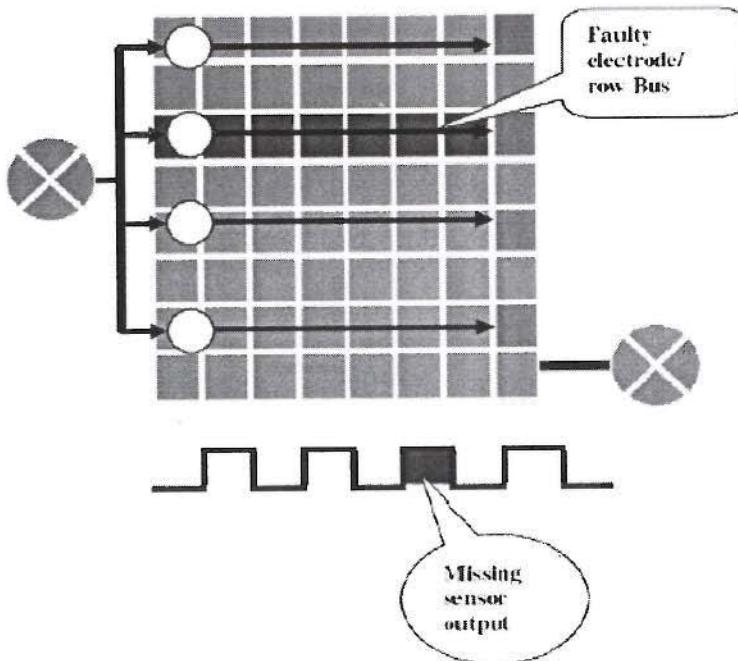


Fig 5: Parallel Row Scan

Two way PARALLEL SCAN METHOD

In order to identify a specific cell location within the 2D array both parallel row scan and parallel column scan is carried out. The rows and columns identified as faulty are marked. Now all the corresponding row column intersection cells are identified to have probable fault. But that may not be the case always. There may be some cells in those intersections that may not be faulty.

1. In such cases for each identified location a test droplet is dispensed from a pseudo source and returned back to the same source that acts as a pseudo sink. Those droplets not returning from the target location are identified and the corresponding target locations are diagnosed as faulty.
2. But this method requires large number of droplets which are equal to the number of identified locations. A definite route plan is necessary to route all the droplets concurrently. The latest arrival time among all the droplets expected to reach back the corresponding pseudo sink gives an estimate of the test completion time.
3. However an additional time of routing each droplet sequentially from each pseudo sink to main sink for capacitive sensing is also to be taken into account.
4. Once all the droplets reach the corresponding pseudo sink (i.e. latest arrival time as per route plan is over) – routing from each pseudo sink to the main sink is started. In this method no cell location remains unreachable as appropriate route plan for all the locations are predetermined taking into consideration the necessary stall and detour).

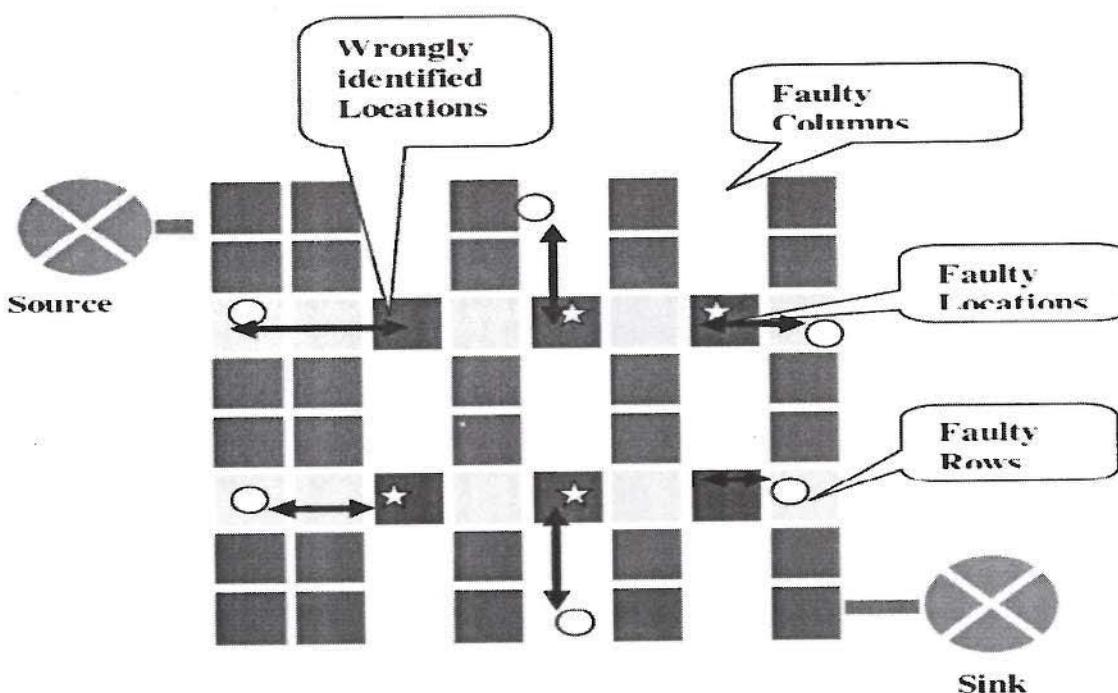


Fig 6: Two way Parallel Scan

Functional Testing of Digital Micro fluidic Biochips

Structural testing of digital micro fluidic biochips targets the detection of physical defects, but it does not guarantee robust execution of target bioassays or the integrity of assay outcomes. Functional testing is needed to detect fluidic malfunctions. Such tests ensure whether or not, the elementary fluidic operations, such as droplet transportation, mixing, incubation, and splitting are reliably executed on the micro fluidic array. Routing test and mixing/splitting test are two important steps in functional testing. We present two procedures for optimal bidirectional routing test and accelerated mixing/splitting test. Compared to previous methods, these procedures need significantly fewer droplet manipulation steps and reduced execution time. The proposed method of functional testing in an $N \times N$ micro fluidic array requires only a constant number of mixing/splitting steps. Further, the test outcome is free from boundary errors related to droplet size that may arise during mixing/splitting test.

Enhanced mixing and splitting test procedure:-

The mixing/splitting test procedure aims at checking the correct functioning of two most extensively used functional modules in a digital micro fluidic biochip, namely, mixers and splitters. To ensure correct mixing and splitting on any group of cells in the micro fluidic array, all four steps i.e., horizontal splitting (HS), horizontal mixing (HM), vertical splitting (VS) and vertical mixing (VM), on each group should be guaranteed. The test procedure proposed in earlier work is performed by carrying out mixing and splitting of droplets for the cells in a row/column of the array individually. Since, mixing and splitting tests in each row/column take two manipulation steps, we need $4N$ manipulation steps to complete mixing/splitting tests for all the cells in an $N \times N$ micro fluidic array. In this subsection, we propose a test procedure in which mixing/splitting tests for all the cells in a micro fluidic array can be performed using a constant number of manipulation steps. In the previous proposal it was proposed that to do split and merge operation at every cell of the chip size of the droplet should be 4X. But we have shown that using 2X droplet size we can perform the same operation and also some problem which may occur while using droplets size of 4X. It has been discussed below;

Volumetric problem with 4X size:-

While splitting a 4X droplet it will first split into two 2X droplets and after that single X droplets. It may happen that while merging a X droplet merge with a 2X droplet which will result a droplet of size 3X. It is impossible to place droplet size of 3X inside a cell.

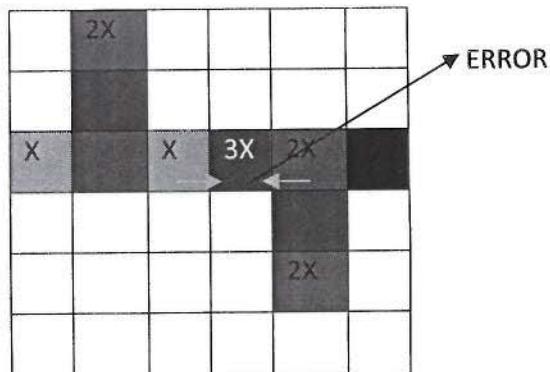
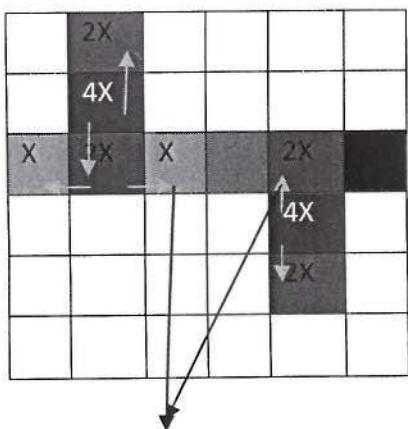
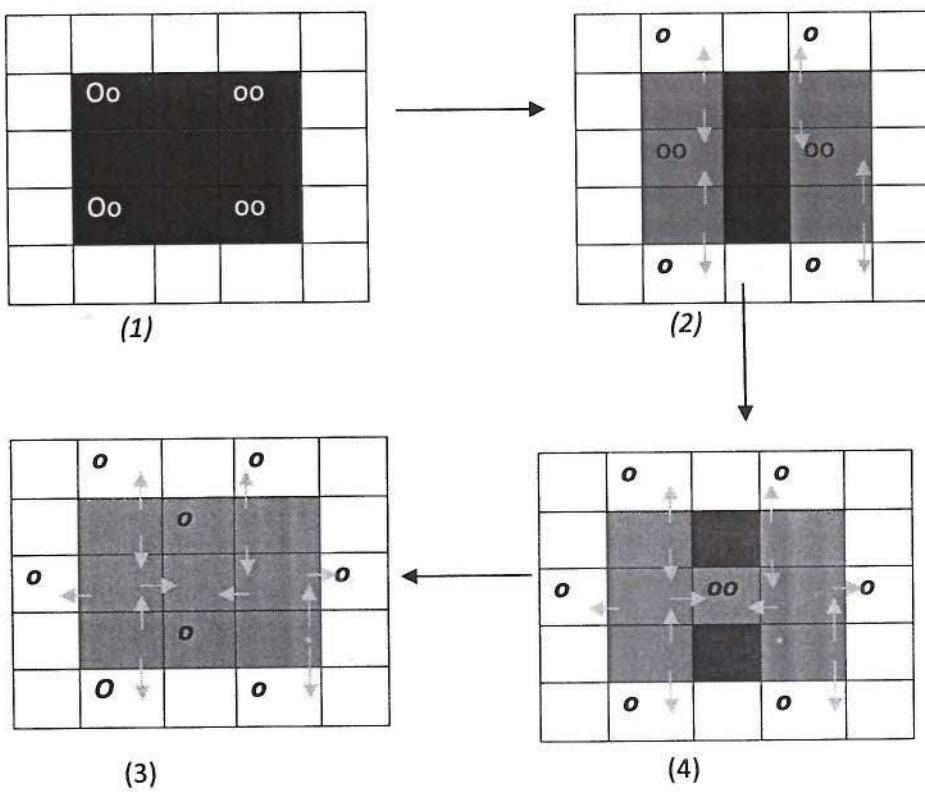


Fig 7: Volumetric Problem in 4x droplets

If X and $2X$ both are in high and the gray cell is in low voltage then both two droplets will try to merge at the gray space.
(BLACK CELL denotes the block).

Merging and splitting using droplet size of $2X$:-



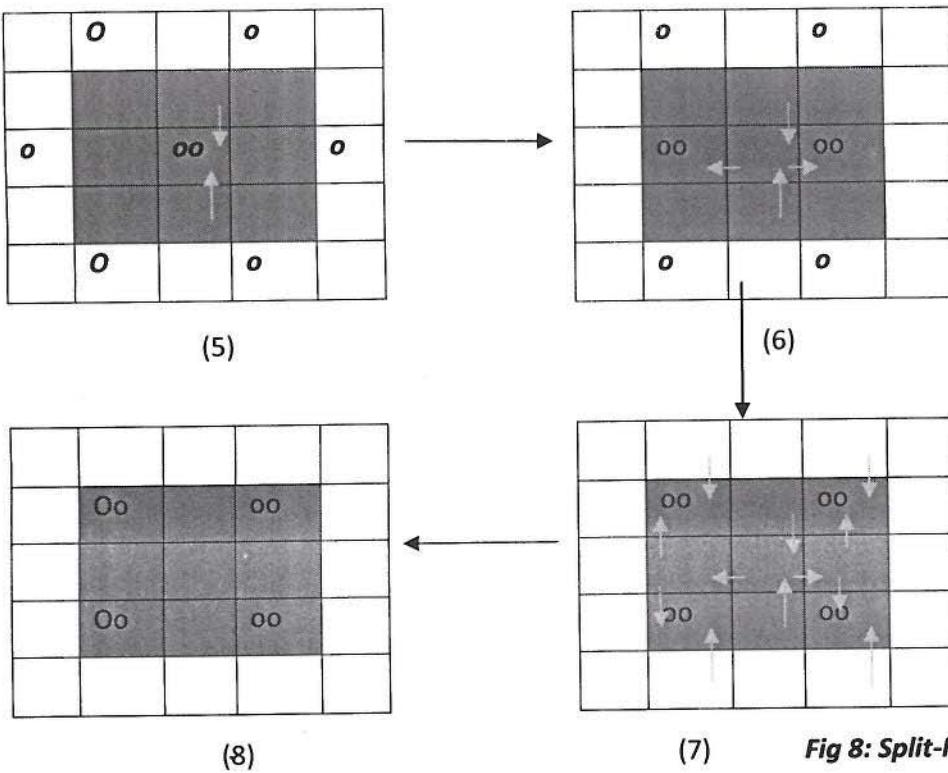


Fig 8: Split-Merge Operations

To cover a 3×3 matrix we need 4 droplets of size 2×2 . As well as to cover a 5×5 matrix we need 9 droplets of size 2×2 .

N x N number of 2×2 droplets cover $(2n-1) \times (2n-1)$ matrix

Accelerating loading/unloading of test droplets:

We have proposed an idea of loading/unloading of droplets of size 2×2 rather than 4×4 . It will take less time to load and unload than a droplet of size 4×4 . After loading we are shifting the droplet to its particular position. While using droplet size of 4×4 we have to shift two blocks to load/unload a droplet but in case of 2×2 we have to shift a single block for loading and unloading.

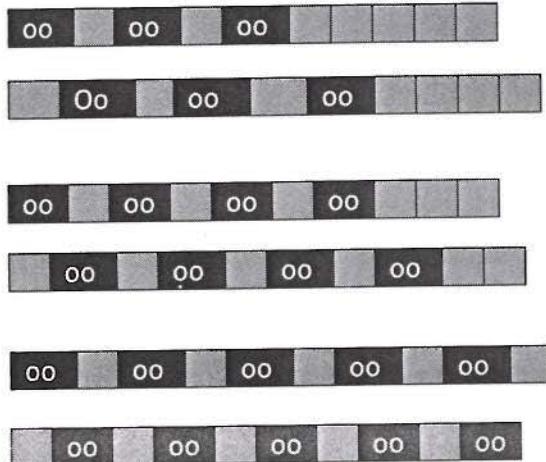
Loading droplets:-



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Unloading of droplets:-

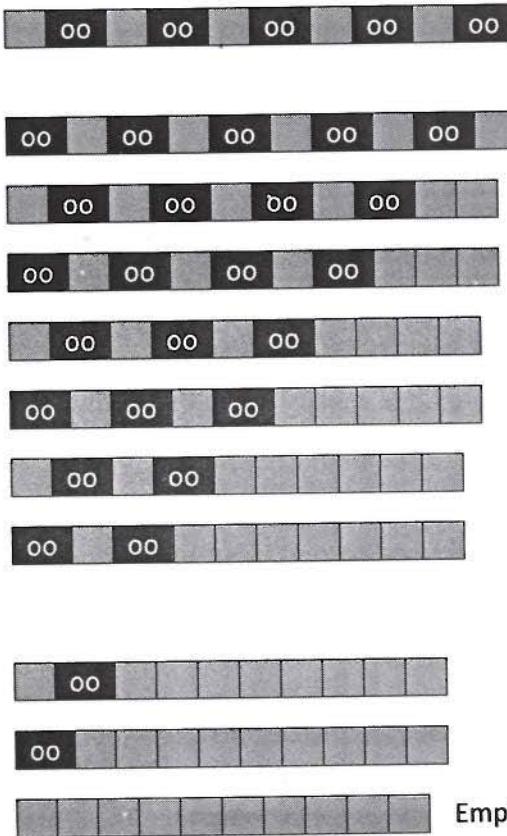


Fig 9: Loading and Unloading of Droplets

Here for loading k droplets in a single row we have to manipulate $2k$ steps. In case of $4X$ it was $[3(k/2)-1]$. Thus $2X$ reduces the manipulation steps.

Customized Route-Path Testing – A New Technique

We have proposed a look ahead testing strategy that handles a given prescheduled bioassay routing operation. The method starts with a predetermined virtual route path and that tests the path specifically for structural faults before the actual transportation of droplet commences. In the process it tries to optimize the test time that enables concurrent routing operation to commence along a path (which has been detected fault free through testing) while testing of other potential routes are continued. The objective of the test method is to optimize the i) overall test completion time ii) overall concurrent route time of the droplets iii) number of source test droplets as well as number of sinks to be used and iv) number of cells to be utilized for both testing and routing of the sample droplets.

Droplet routing in DMFBs

The main objective of droplet routing is to transmit all the droplets from their respective sources to targets within a 2D grid array while fulfilling all the constraints encountered in the route. An efficient routing schedule (virtual route) is often required to be developed to provide an optimal routing in terms of objectives such as latest arrival time and total electrodes utilization. Droplet routing problem in DMFBs is typically modelled in terms of a 2D-grid (Figure 2). For each droplet, there exists a set of source grid locations, a set of target grid locations, and (optionally) a set of mixers. Each source-target combination is defined as a net. A 2-pin net has a single source and single target. Multiple sources, mixers and a single target form a multi-pin net. A combination of two Sources, one Mixer and one Target is a 3-pin net.

In cases of 3-pin or multi-pin nets, droplet merging is desired at specific locations called mixers. Several microfluidic modules may exist for mixing, splitting, storage, dispensing and other operations placed on the array. These are considered as the *Hard Blockages*. In order to avoid conflicts between droplet routes and assay operations, a segregation region is defined around the functional region of such microfluidic modules to isolate the droplets from active microfluidic modules during routing. Moreover, there are possibilities of intersection or overlapping of droplet routes during their concurrent routing in time-multiplexed manner. To avoid such undesirable behaviours following fluidic constraints are introduced.

Fluidic Constraints

Let d_i at (x_i^t, y_i^t) and d_j at (x_j^t, y_j^t) denote two independent droplets at any given timestamp t . Then, the following constraints, called *Fluidic Constraints* should be satisfied for any time t during routing [22]:

Static constraint: $|x_i^t - x_j^t| > 1$ or $|y_i^t - y_j^t| > 1$

Dynamic constraint: $|x^{t+1}_i - x_j^t| > 1$ or, $|y^{t+1}_i - y_j^t| > 1$

or, $|x^{t+1}_i - x^t_i| > 1$ or $|y^{t+1}_i - y^t_i| > 1$

This implies that for any droplet at location (x, y) , the locations $(x+1, y), (x-1, y), (x, y+1), (x, y-1), (x+1, y+1), (x+1, y-1), (x-1, y-1)$, and $(x-1, y+1)$ are prohibited for any other droplet to enter at timestamps t and $t+1$ in order to maintain these fluidic constraints. Hence, all the locations adjacent to (x, y) as stated above form a *Critical Zone* (Figure 2) for any droplet at (x, y) at timestamp t . A predetermined time limit called the *Timing Constraint* defines the maximum allowed transportation time for a given set of droplets.

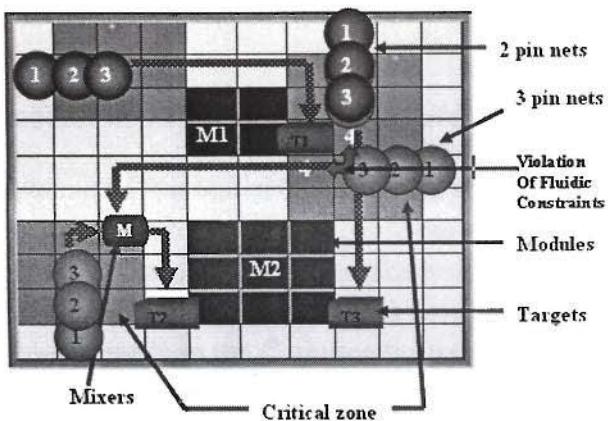


Fig 10: 2-pin and one 3-pin nets in a 2D Grid Array; Critical zone for sources is shown by the GREY shaded region. Hard Blockages are also shown by BLACK regions. All sources instantaneous position at timestamp 3 is given. Violation of fluidic constraint occurs at timestamp 4.

The Work Plan:

1. Obtain the virtual route path for the given droplet placement.
2. Draw an undirected line graph with vertices showing the source, targets, mixers for each droplet and the corresponding connections in the route as edges. Crossovers are indicated by intermediate nodes.
3. Perform a depth first search for all connected components of the line graph to form a depth first tree for each such connected component.
4. For each depth first tree obtain the longest path covering maximum number of nodes (including the intermediate ones) starting from one pendant node and ending to the other.
5. Insert one 2x droplet through each end of this longest path and arrange to split it at the first intermediate node encountered during traversal through the path.
6. Perform traversal through the longest possible walk within longest path and exit each 1x droplet through the corresponding pendant nodes posing as exit point.
7. Check for the edges and vertices in the line graph that is not traversed at all and form a residual graph deleting all pendant nodes already traversed and the intermediate nodes whose all edges are covered.
8. In this residual graph again find the longest path and repeat step 5-7 and continue until all edges are traversed.

9. If however in these longest paths if it is found that there exists no branching use a 1x droplet only through one entry point and no splitting is required.
10. Finally on obtaining the scheduled route for testing optimize the dispenser location based on the exit points derived on a given side maintaining the dispenser constraints as stated later.
11. Perform the routing (according to the scheme stated in section 8) of test droplets assuming timestamp of 1 for routing and 2 for splitting.
12. Compute the latest arrival time among all these test droplets – the total number of actual cells tested and the number of additional cells traversed for completion of testing.
13. Note down the total volume of test droplets required as well as the number of dispensers required to perform testing of a given layout.

Determination of Virtual route path

Hightower's line search based routing algorithm [23] is applied to compute the shortest distance between each source and target (2-pin net). For a 3-pin (multi-pin) net, the same algorithm is applied to compute the shortest distance between each source and mixer, and between the mixer and the target. If x_i is the distance between the i^{th} source Sx and mixer of a 3 pin net and y_i is the distance between the i^{th} source Sy and mixer of the same 3 pin net and z is the distance between mixer and the target, the [maximum of $(x_i, y_i) + z$] is computed as total distance for each 3 pin net. This maximum distance serves as an estimate of the droplet route length, and helps to resolve conflicts in concurrent routing of multiple nets. The procedure for determining the estimated distance based on [23] is described below.

- Line segments are generated from each source and target (mixer) that corresponds to a single droplet.
- Both horizontal and vertical line segments are generated from each source and target point(location).
- Whenever the source and sink line segments intersects – the exploration phase ends and single or multiple paths are found.
- If a line probe intersects an obstacle, from each such intersection point an escape point is formed. From this escape point a new line probe is generated perpendicular to previous one.(Figure 3)
- The same exploration phase is repeated until at least one source originated probe meets at least one sink originated probe -- that implies a routing path is established between the source and the corresponding sink. (Figure 4)
- Time complexity of this algorithm is $O(L)$, where L is the number of line segments generated in the exploration process.

In selection of virtual route paths there may be multiple paths between each source and target for any given net. Here preference has been given to all those paths that minimizes the cross contamination between different nets in order to prevent the wash droplet routing overhead.

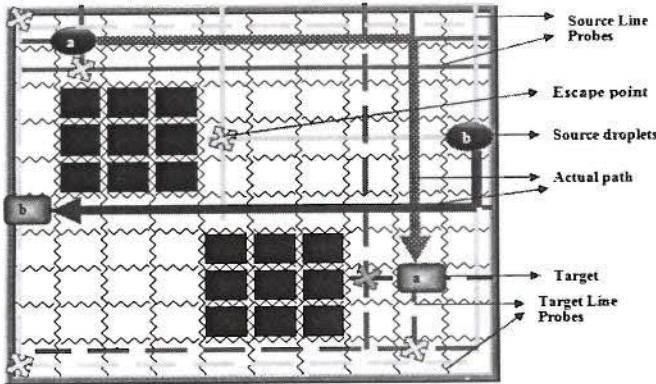


Fig 3: Determination of virtual route path using Hightowers line probe algorithm. Selected paths are shown with Thick arrows

Proposed algorithm for formation of Test schedule

Problem formulation

A $m \times n$ - 2D planar array of electrodes are given. Given a specific layout for x number of 2 pin and y number of 3 pin nets. The virtual route path with minimum number of cross over is obtained using Hightower's Line probe Algorithm [23] as stated in section 6. Let the total number of route paths obtained is

$$k \leq x+y.$$

Our target is to obtain an efficient test schedule that uses :-

- p number of test droplets
- d number of dispensers
- Maximum test completion time as T .
- Number of cells covered is r .

to ensure that - p number of test droplets cover all the e edges of k paths simultaneously within time T , such that

Objective 1 – number (test droplets) = p is optimum

Objective 2 – number (detectors) = d is minimum

Objective 3 – Test completion time T is minimized

Objective 4 – number of cells covered for testing r is minimized.

Predefined Constraints

1. Each test droplet volume is maximum $2x$ as a single cell can hold maximum $2x$ volume of droplet.
2. Number of test droplet inserted on any of the four sides of the 2D grid should not exceed Two. This value is heuristically assumed to avoid congestion.
3. Maximum number of detectors on each side is One – to optimize cost as dispenser costs are quite high. This implies that number (dispensers) ≤ 4 .
4. Fluidic constraints as stated in section 4 is to be followed while routing of test droplets.

The Algorithm

Input:

1. A two-dimensional array of electrodes placed over a 2D array of microfluidic biochip.
2. A set of modules already scheduled as per the operational requirements and already placed within the 2D array.
3. A set of sub-problems with source and target locations predefined in the testbench for 2-pin nets, and multiple sources, mixers and corresponding target locations for multi-pin nets.

Output:

A optimized test schedule with optimum number of test droplets and droplet sinks/detectors showing the optimized route path for the test droplets.

Procedure:

1. Using **Hightower's Line probe algorithm** the virtual route path for each net with minimum number of cross contamination is estimated.
2. Let there are total k such route paths for all the nets scheduled for routing.
3. based on these k number of virtual route path a line graph $G=(V,E)$ is drawn where each vertex $v \in V$ represents a source or mixer or target location or any cross point at which two route paths intersect.
Each edge $e(u,v) \in E$ represents the existence of a routing path between two vertices u and v as obtained from the route plan.
each edge e_i is assigned a field $\text{Trav}(e_i)=0$.

Each vertex v is assigned a field $\text{Mark}(v)=\text{New}$

4. let there be m number of connected components for the line graph and they may be denoted as G_1, G_2, \dots, G_m .
5. **For $i = 1$ to m**
 For $G_i = (V_i, E_i)$
 1. Perform a **depth first search** starting from any one pendant node – to form the depth first tree.
 2. Compute the longest path in the depth first tree
 In terms of highest number of nodes (both pendant and crossover) covered within the path starting from one terminal node v_x and ending to another terminal node v_y .
 3. These two end terminals serve as entry point of two test droplets for traversal within G_i .
 4. Initially we assume the volume of each such droplet to be maximum as $2x$.
5. Compute weight w_j for each edge $e_j \in E_i$ based on the number of cells covered to reach from u to v where u and v are the terminal points of e_j .

6. start traversal from v_x – once any crosspoint is reached the droplet is split into two and move along the edges having higher weight if $\text{Trav}(e_i)=0$. This is applicable if the cross-point node has ≥ 2 number of branches.
 7. All the newly explored edges are marked as $\text{Trav}(e_i)=1$. All the newly explored vertices are marked as $\text{Mark}(v_i) = \text{discovered}$.
 8. During such traversal if at any crossover point it becomes necessary for choice of a path between multiple explored edges ($\text{Trav}(e_i) \geq 1$) – the edge with minimum weight is selected to save overall time for traversal and increment $\text{Trav}(e_i)$ by one.
 9. Repeat the same traversal for the droplet entered through v_y (steps 6 -8).
 10. If for any crossover vertex v has all its edges having $\text{Trav}(e) \geq 1$ then compute $\text{Mark}(v) = \text{Finished}$.
 11. While traversal from any entry point if it has been found that no splitting of the test droplet is necessary as it travels through a single straight path (without any branching)- assign volume of $1x$ to that test droplet.[as no splitting is required].
 12. Once the traversal of v_x and v_y is over – delete all nodes with $\text{Mark}(v) = \text{finished}$. Delete all the edges connecting such nodes .However nodes with any of its edges not discovered are not deleted – as well as edges connecting any two such nodes are not deleted.
 13. A residual graph G_{IR} has been formed.
 14. Repeat steps 2 to 13 iteratively until all edges of G_i is not traversed.
 15. Note the total volume of test droplets required to be inserted and also mark the exit points on each side of the Grid.
- Optimization of detector /sink location**
16. On each side on completion of final routing check the exit point Q at either side with highest arrival time.
 17. Compute the arrival time of all other test droplets exiting through that side to the aforesaid exit point.
 18. If any of the droplet d_j has arrival time larger than the one at Q choose at a symmetrically middle location between earlier Q and the exit point of d_j .
6. Now compute the total number of test droplets p as well as total number of detectors/sink d required for completion of the test schedule.

Concurrent routing method of Test droplets

Step 1. Based on the test plan start routing all test droplets concurrently along the assigned path obtained from the test plan. Routing should be carried out according to the **algorithm** stated below.

Step 2 . The arrival of each test droplet in a sink indicates the clearance of corresponding segments of a path specific for a corresponding source. However the sequence of arrival of all the test droplets to a given sink is noted in order of their arrival time. This gives information about all those segments traversed by the test droplets cleared of obstacle – and the arrival sequence of each test droplet to the sink corresponds to the partial path coverage of the corresponding source droplets.

Step 3. Based on these arrival sequence information for all the sinks –a source to detector information table is prepared. Each source to target route is said to cleared once all the test droplets corresponding to the path segments for the given source droplet arrive to the sink.

Step 4. Compute the test completion time for each test droplet at the sink. Also compute the total number of cells r utilized in the process as well as actual number of cells covers the route path r_a . The number of additional cells covered for inserting the test droplets and dispatching it to the sink after covering the destined segments are obtained as $(r - r_a)$.

Routing Algorithm for concurrent route

1. Based on the virtual route path as obtained in section 6 when two or more test droplets are routed concurrently they are first sorted in order of the computed path distance from respective entry to exits .These distances are termed as ***Hightower-Distance*** for each sample droplet.

2. During concurrent routing of several droplets, there is a possibility of collision or overlap of paths of multiple droplets. In such a case, priorities are given in favour of the droplets with larger ***Hightower-distance***. Whereas droplets with lower priorities are either stalled or detoured through a different path already included in the route plan (if that allows for no further increase in critical time – which counts for the latest arrival time for the droplet with largest Hightower -Distance).

[Type text]

Test Results

In_vitro_1

Sub-problem No.	No. of Droplets Used		No. of Dispensers Used	Test Completion Time (L.A.T.)	Reservoirs Used	Additional cells Used
	x	2x				
1	2	1	2	32	2	3
2	2	1	2	33	2	13
3	3	0	2	24	2	14
4	1	0	1	14	1	8
5	1	1	1	28	2	3
6	4	0	3	20	3	10
7	0	1	2	21	1	11
8	2	0	2	15	2	4
9	2	0	2	15	2	4
10	1	0	1	15	1	5
11	1	0	1	16	1	5

[Type text]

In_vitro_2

Sub-problem No.	No. of Droplets Used		No. of Dispensers Used	Test Completion Time (L.A.T.)	Reservoirs Used	Additional cells Used
	x	2x				
1	2	0	2	16	2	7
2	3	0	2	16	2	2
3	2	2	2	39	2	4
4	1	1	2	24	2	12
5	3	0	3	10	3	9
6	2	0	1	17	2	2
7	1	0	1	15	1	2
8	1	2	2	15	2	5
9	4	0	2	25	2	5
10	1	0	1	7	1	2
11	1	0	1	15	1	2
12	2	0	2	8	2	6
13	2	0	2	16	2	6
14	1	0	1	15	1	2
15	1	0	1	6	1	0

Conclusion

Our main objective in this project is to create a test simulator for microfluidic biochips. So we have to address various kinds of faults that can occur in a biochip while traversing the array of electrodes. So initially we concentrated on the particular aspect of **traversal of the biochip through electrode array where there may be obstacles**. Further we have also carried out **functional testing** of biochip array in detail and also devised **an efficient algorithm for rout path testing**, and also carried out **concurrent testing** for various diverse test cases. The works we have carried out so far would contribute to the completion of this ongoing research project.

References

- [1] F. Su, S. Ozev and K. Chakrabarty, "Test planning and test Resource optimization for droplet-based microfluidic systems", JETTA, vol. 22, pp. 199-210, 2006.
- [2] S. Datta et al., "Efficient parallel testing and diagnosis of digital microfluidic biochips", ACM Journal on Emerging Technologies in Computing Systems, vol. 5, article 10, 2009.
- [3] D. Davids et al., "A fault detection and diagnosis technique for digital microfluidic biochips", IEEE International Mixed- Signal, Sensors, and Systems Test Workshop, 2008.
- [4] D. Mitra et al., "Accelerated functional testing of digital microfluidic biochips", IEEE Asian Test Symposium, pp. 295- 300, 2008.
- [5] F.Su,S.Ozev,K.Chakraborty, Concurrent testing of digital microfluidics-based biochips", ACM Transactions on Design Automation of Electronic Systems (TODAES), vol 11 Issue 2, April 2006 .
- [6] K.Chakraborty, "Towards fault tolerant Digital Microfluidic Lab-on-chip: Defects, Fault-Modeling, Testing and Reconfiguration" Proc of IEEE Biomedical Circuits and Systems Conference, BioCAS, 2008, pp-329-332.