Iterative Parallel Test to Detect and Diagnose of Multiple Defects for Digital Microfluidic Biochip

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***Abstract*—Digital microfluidic biochip is a revolutionizing platform to execute complex bioassay operations concurrently. Dependability is an important feature of digital microfluidic biochip which is used for many safety-critical applications, such as point-of-care health assessment, air-quality monitoring, and food-safety testing. Therefore to ensure the proper functionality of the biochip a robust offline and online testing is required. Testing can be done before manufacturing or concurrently with other bioassay operations. In this work, we are presenting an efficient parallel testing and diagnosis scheme to reveal the location of all the faulty electrodes. Most of the algorithms present in the literature are mainly focused on single fault identification. But the diagnosis of biochip with multiple faults is not addressed properly. Even some of the algorithms have incorrectly classified the non faulty electrode as a faulty electrode. Thus in this paper, we are mainly focusing on the detection of multiple faults, correctly and efficiently. Moreover, this testing method is capable to check the given biochip with other concurrently running bioassay operations.**

***Index Terms*—Biochip, electrowetting, Peripheral test, RD test, DR test, Unit test, Bypass Test, fault model, test time**

I. Introduction

Over the last decade, Digital Microfluidic Biochips (DMFBs) caught much attention to replace the cumbersome and expensive laboratory equipment. These devices are also referred to as lab-on-a-chips [1] which enables miniaturized analysis systems for biochemical applications, such as point-of-care clinical diagnostics [2] and DNA sequencing [3]. These systems of integrated circuits by combining the microstructures with solid-state electronics work on different domains like electrical, mechanical, and fluidic energy domains. Due to these mixed energy domains, digital microfluidic biochips have exhibited some unique failures which are unlikely from digital circuits. These new kinds of integrated automated system offer higher sensitivity and cost effectiveness because of smaller sample and reagent volumes. It has fast turn-around time for analysis, higher levels of system integration and fewer chances of human error. Microfluidic biochips are divided into two types:

1) **Continuous Flow Biochip:** Continuous Flow Biochips are based on the continuous flow of fluid [4] through permanently etched micro capillary channel and the different fluidic operations are controlled by using a large number of micro-pumps and the micro-valves [5] externally attached to it. But it is difficult to integrate such a large number of micro-pumps and micro-valves as well as controlling different fluidic parameters for proper operations of the biochip.
2) **Digital Microfluidic Biochip:** Digital Microfluidic Biochips are the type biochips which works with discrete droplets. The nanoliter (μl) droplet containing the biological samples moves on the patterned arrays of electrodes and it is manipulated using the phenomenon of electrowetting actuation [6]. Moreover, these new kinds of biochips provide more flexibility and due to its two dimensional scalable system architecture, it can offer better controllability compared to the early generation biochips. It can also reconfigure dynamically which enables us to remap [5] any fluidic operation to any part of the biochip. Hence, the DMFB provides a wide variety of applications like parallel DNA analysis and real-time molecular recognition etc..

Recently different sensors have been integrated with DMFB for analysing the activity of the biochips [7]. For example, integrated waveguides [8], capacitive sensors [9], or CCD cameras [10] are used to monitor bioassay operations. The massive parallelism of multiple bioassay execution steadily increased the system complexity and also increased the integration complexity of digital microfluidic biochips. Hence the increased chip density automatically reduces the yield rate. These inexpensive processes and materials are explored by the device manufacturers for lowering the cost of disposable device manufacturing.

The biochips are used for many safety-critical applications like health assessment and screening for infectious diseases where the dependability is a very much important attribute. Moreover, when the biochip is used for pharmacological procedures like drug design and discovery then these processes demand high accuracy in the precision levels. Some of the errors are produced in the biochip due to unpleasant and harsh operating environment. During field, operation absorbed biological samples (e.g., proteins) residue may contaminate with other particles which causes incorrect sample preparation. Hence a cost effective test plan is required due to the competitive global market of disposable biochips and which can also ensure the chip condition for further operations.

Hence biochip testing caught much attention in past few years. In broader sense testing can be categorized into two

types: structural testing [11] and functional testing [12]. To find out the physical defect in any electrode we have to perform structural testing whereas functional testing is required to check a group of cells which is collectively known as "module", is working correctly or not. A correctly working cell during transportation may produce an incorrect result when the same cell is used for splitting operation. Thus structural testing never ensures the proper functionality of a module. Most common DMFB defects can be detected by routing one or more test droplets across the chip and recording their arrival time at the destination.

The contributions of this paper are as follows:

1) We propose a testing and fault diagnosis method that can detect multiple faults with reduced test time.
2) This method is applicable for both offline and online testing.

The sequel of the paper is as follows. Section III describes the basic working principle of DMFB. Section IV presents the motivation of the proposed work. Section V describes the fault model as well as the problem definition. Working principle of the proposed method is discussed in Section VI. Next, we have described the fault diagnosis technique in Section VII. Simulation results and the comparison with other methods is shown in section VIII. Finally, Section IX draws the conclusion.

II. Overview of Digital Microfluidic Biochips

A digital microfluidic biochip is a patterned array of individually controllable electrode. The conductive and polarizable droplet is sandwitched between two parallel glass plates. Using the principal of electrowetting actuation [6] a droplet can be moved from one electrode to the adjacent electrode. The droplet volume is slightly larger than the pitch of the electrode so as to maintain sufficiant overlaping between the drolet and the neighbouring electrode. Consider the Fig. 1), where the droplet's curent position is electrode 3. In order to move the droplet to the neighbouring electrode a time varying voltage is applied to the adjacent electrode, whereas electrode 3 is deactivated at the same time. Hence a surface energy gradient has established which forced the droplet onto the charged electrode. Capacitive sensing circuit (shown in Fig. 2) is used to check whether a droplet is present at the sink or not. In a DMFB droplets are dispensed from a reservoir and then the droplets are routed, splitted, merged and mixed using electrowetting actuation [6] for different biochemical operations.

III. Literature Survey

Different types of test techniques of DMFBs have been reported in this literature. In [13] authors have proposed a unified test methodology for detecting a fault by tracking the droplet electrically. Multiple test planning methodology referred as "parallel scan-like test" has been proposed in [14] where multiple droplets are parallelly transported to know the exact location of the faulty cell. To reduce the incurred test time another parallel test planning method is proposed in

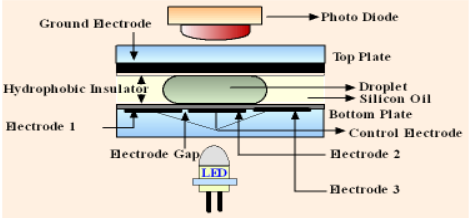

Fig. 1. Schematic diagram of digital microfluidic biochip

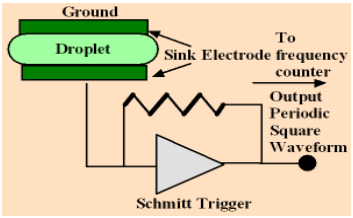

Fig. 2. Schematic diagram of Capacitive-sensing circuit.

[15]. Various types of fault detection techniques have been proposed in [11], [16], [17]. Considering of different types of physical constraints an ILP based model is presented in [18]. In [19], [20] authors have presented the fault model and the fault simulation method for continuous flow.

A graph based model presented in [21] where the given problem is mapped to the Hamiltonian cycle problem which is known as $NP - complete$ [22]. To improve the overall test time a Monte Carlo simulation based heuristic solution is presented in [21]. In [23] authors mapped a DMFB into an undirected graph and determined a Euler path to visit all the nodes of the graph. However, this incurred a high testing time. A single fault detection technique has been proposed in [23]. To improve the test and diagnosis time another method for rectangular biochip is presented in [24].

In [13], [25] authors presented the techniques for defect classification, test planning, and test resource optimization for DMFB. In [18] authors formulated the test time for any arbitrary layout of DMFB. A combined approach of synthesis, testing and reconfiguration has been proposed in [1]. In [12] a bidirectional routing test plan for functional testing is explained. However, an effective test and diagnosis method is required to implement it at a low cost.

IV. Motivation

To detect electrode faults one or more droplets are guided from a specified source to sink following a predefined test path. Now after a certain time period if it fails to reach the destination then we consider that routing path as a faulty path, although this condition is not sufficient for fault identification. In *Vertical Stripes Algorithm* [11] each droplet is routed using predefined the routing path which is shown in Fig. 3(A) using the Dotted line. Suppose *stuck at 0* fault exists in electrode 7 as shown in Fig. 3(A) and we have to identify this error. Due to *stuck at 0* the fault in electrode 7, the test droplet will temporarily stuck at electrode 8. After a few time cycles

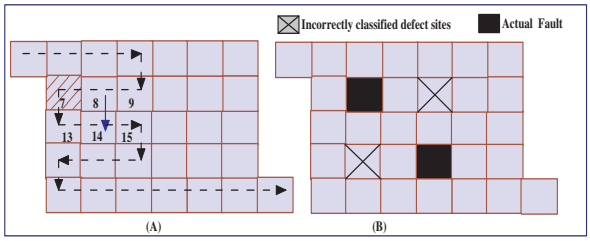

Fig. 3. (A) Unidentified faulty electrode using Vertical Strips Algorithm (B) Incorrectly classified faulty electrodes using Parallel Scan Like Test

when the electrode 14 which is the neighbour of the electrode 8 is actuated then the stucked droplet automatically moved to electrode 14. As a consequence, the droplet again came back to its actual routing path and finally reached to the sink and it is detected. Hence the fault that exists in the electrode 7 remains undetected. This error is undetectable due to the improper routing path design. The same situation also arises for the other algorithms discussed in [11].

One of the major drawbacks of the parallel scan like [14] test is identifying a non-faulty electrode as a faulty electrode. Just examining only the failing rows and columns it is difficult to identify medical defects. Because intersections with failing columns and rows are possible for both the defect sites as well as defect free electrodes as shown in Fig. 3(B). To overcome these two major drawbacks, here we propose a methodology named *Iterative Test Method (ITM)* to detect multiple faults. Using this model we can identify the correctness of the chip as well as the location of the faulty cell(s).

V. Fault Model and Problem Definition

Like microelectronic circuits, DMFBs may be subjected to failure when its operation does not match with the specified behaviour. Different kinds of fault models are used with a layer of abstraction in order to detect the causes behind the defects. In this paper, we are mainly focusing on two types of faults, electrode open faults and electrode short faults [17], [26], [27]. Electrode open fault occurs due to the lack of connection between the faulty electrode and the voltage source. When a droplet stuck between two adjacent electrodes an electrode short fault occurs. In this paper, we are using *droplet tacking* method to know the presence of electrode open fault(s). If all the specified number of droplet detected at the sink then the biochip is in good condition else the biochip is faulty. Hence further processing is required to know the exact location of the faulty cell(s). The proposed fault detection methodology discussed in Section VI uses an iterative method to detect the multiple numbers of faults within considerable time. The problem is formulated as follows:

Given: Any rectangular chip size of $M \times N$ biochip.

Objective: Detect and determine the exact locations for K numbers of faults present on this biochip.

VI. Iterative Test Methodology

In this section, we present an efficient fault detection and diagnosis method based on parallel droplet testing. One way

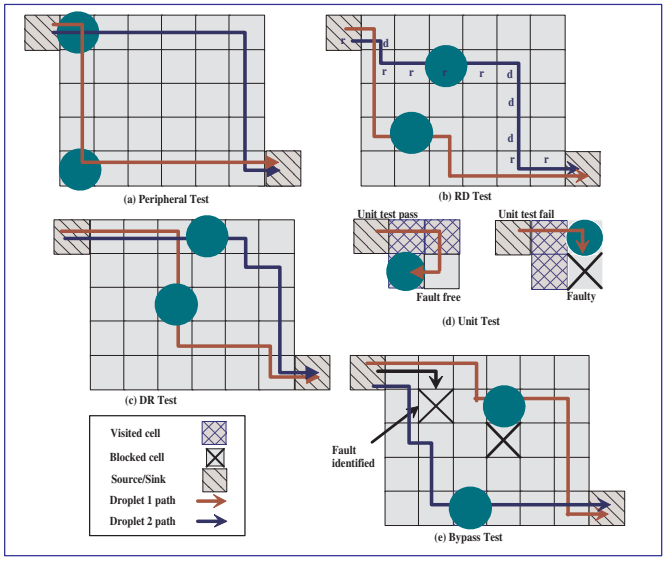

Fig. 4. Steps of the proposed method

to detect faults is to route the test droplets between every pair of adjacent electrodes which can take huge time for testing. One of the primary objectives of the testing is to minimize the overall test time. Thus without loss of generality, droplets are dispensed from the reservoir located at the top left corner of the chip to traverse a pre-specified routing path and reach to the sink located at the bottom right corner. In this work, we consider only one source and sink pair for the DMFB. The capacitive sensing circuit is used for getting test result at the sink. In this parallel droplet scheme, sufficient gaps between droplets need to be maintained for avoiding accidental merging.

The movement of the droplets from source to sink is described by the alphabet set $\{l, d, r, u\}$. Here l, d, r, u denote a droplet movement to the *left, down, right* and *up* respectively with respect to a position. Our test methodology comprises of five individual steps. The first step is known as the *peripheral test* which is necessary to check the correctness of the boundary electrodes. The second step is used to make sure that all the cells of the biochip are in good condition. The third step is used to diagnose a single fault which is present in the biochip. Next two steps are required to detect multiple faults in the biochip. In Algorithm 1 our method (*Iterative Test Method (ITM)*) is discussed and the basic steps followed in this algorithm are discussed below,

1) **Peripheral Test:** Two separate test droplets are used to visit all the boundary cells shown in Fig. 4 (a). One droplet is used to visit all the cells located at the top and the right side and the other droplet is used to visit the remaining boundary cells. Droplets are dispensed from the source with sufficient time cycles gap to avoid accidental merging.
2) **Right Down (RD) Test :** Two iterations are carried out to test the biochip. For the first iteration, we place

the droplets in the even-numbered rows only, which is shown in Fig. 4 (b). Then in the next iteration, we place the droplets in the odd-numbered rows only. The droplet trajectory path is described by the string $rd^{i-1}r^{n-i}d^{m-i}r^i$, where $1 < i < m$. Here, i denotes the trajectory path and it is counted from the top. For example, consider the biochip of size 5×6. For $i = 2$, the string value is $rd^{2-1}r^{6-2}d^{5-2}r^2 = rdr^4d^3r^2$ shown in Fig. 4(b). If any droplet failed to reach at the destination within the speculated time then the cells present in this routing path is added to the $Faulty\_List(F)$.

3) **Down Right (DR) Test:** Here also two iterations are carried out like the previous one. For the first iteration, we place the droplets in the even-numbered columns only. Same way, in the next iteration we have to place the droplets in the odd-numbered columns only. The droplet trajectory path is described by the string $r^{n-i+1}d^{i-1}r^{i-1}d^{m-i}r$, where $1 < i < n$. Here, i denotes the trajectory path and it is counted from the right. Like, *RD Test* unvisited cells are added to the $Faulty\_List(F)$.
4) **Unit Test:** This test is recommended to diagnose multiple faults. We have to test all the cells from the $Faulty\_List(F)$ for exact fault localization. This test is coupled with another test known as the *Bypass Test* which is discussed next. We select a cell x from the F and the x has at least three visited neighbours. Created a path P from source to x and x to destination. Now it is recommended that all the cells in this P are visited cells except the cell x. If we use a droplet to follow this path P, then there are two possibilities; either the droplet will be able to reach at the sink or unable to reached at sink. If the droplet fails to reach at the sink then x is the faulty cell and the *Bypass Test* is needed. Now, if the droplet reaches at the sink then x is not a faulty electrode thus it removes from the F.
5) **Bypass Test:** This test is coupled with the previous stage *Unit Test*. After identifying x as faulty we bypass x and visit all the unvisited cells exist in the same row or the same column. Two separate droplets can be used to visit the unvisited cells. The first droplet will visit only those unvisited cells that exist in the same row with x and the second droplet will visit all the unvisited cells in the same column with x. If the droplet is detected at the sink all the unvisited cells in this path all marked as a visited cell and remove from the $Faulty\_List(F)$. Otherwise, further testing is required. We repeat the last two steps (Unit Test and Bypass Test) multiple times until F is empty.

VII. Diagnosis of Multiple Defects

This section describes Algorithm 1 to diagnose multiple defects with an example. Consider a 5×6 biochip as presented in Fig. 5. We assume that the fault locations are $\{(2,2),(3,4)\}$ and we have to identify these two faults using ITM. Hence first, we apply the peripheral test to know the correctness of the boundary cells and find that there is no issue in the boundary cells. We apply the next two steps that are RD Test and DR Test and find that some of the cells are unvisited. Hence, we are adding these unvisited cells to the Faulty_List (F) that contains the positions $F = \{(2,2),(2,4),(3,2),(3,4),(3,5)\}$. Then we pick up the first element that is $x = (2,2)$ from F and apply the unit (step 4) test on x. We create a path for test droplet which will pass through this x and if it is detected in the sink then x is fault free. Here x is detected as faulty cell hence Bypass Test is required. Thus we have visited all the unvisited cells in F which are in the same row or same column of x and found that the test droplet has successfully visited {(2,4),(3,2)}. Hence x is identified as a faulty electrode and the new updated set is $F = \{(3,4),(3,5)\}$. As F is non empty, we follow the last two steps iteratively to find the other faulty electrodes. In this way, we can find the exact locations of the electrodes from a given biochip.

Algorithm 1: Algorithm Iterative_Test()

```
Input  : M × NBiochip
Output: Faulty Set F_List
if Peripheral Test fails then
    exit from Iterative_Test()
else
    foreach Unvisited Row r do
        RD Test
        if r fails the RD Test then
            add all the unvisited cells of this trajectory to F
        end
    end
    if F is empty then
        biochip is in good condition and exit from Iterative_Test()
    end
    foreach Unvisited Column c do
        DR Test
        if c fails the DR Test then
            add all the unvisited cells of this trajectory to F
        end
    end
    foreach element in F do
        choose an element x which atleast three unvisited neighbours
        if do Unit Test on x then
            x is not faulty and remove from F
        else
            x identified as faulty
            remove x from F and added to F_List
            remove all the cells from F satisfying the Bypass Test
        end
    end
end
```

A. Testing time formulation

We will calculate the total off-line test time for a $(M \times N)$ biochip using the proposed method ITM. Testing in off-line means all the biochip cells are available for testing. We perform first two steps (Peripheral Test and RD test) only to know whether the chip is in good condition or not. To find out the total test time (T) we should know the total number of cells covered by each droplet. If the number of cells in that path is K then the number of time cycles required to reach source to sink is $K - 1$. In ITM all the droplets covered the same number of cells, that is $K = M + N - 2$. Now we required $D = 2 + (M - 2) = M$ number of droplets up to RD test. We know that at least 3 cycles gap is required between two

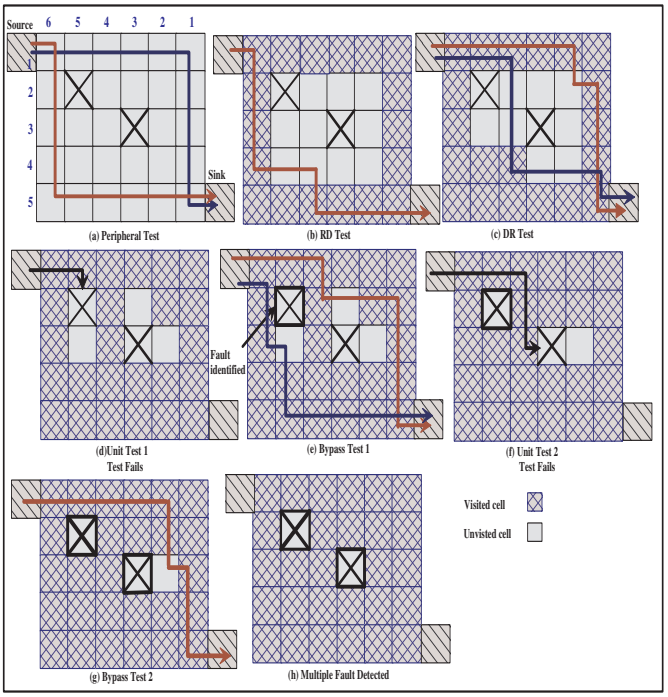

Fig. 5. ITM Method for Multiple Fault Diagnosis

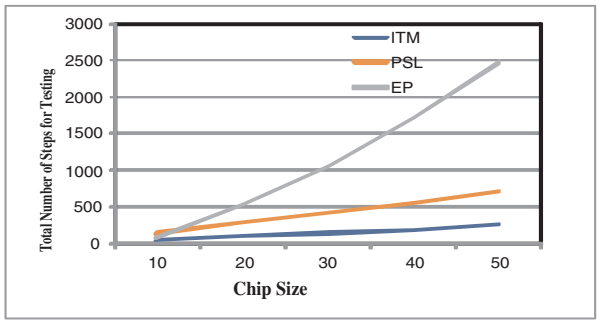

Fig. 6. Comparison of the test time among proposed method ITM, Parallel Scan Method (PSL), Euler Path (EP)

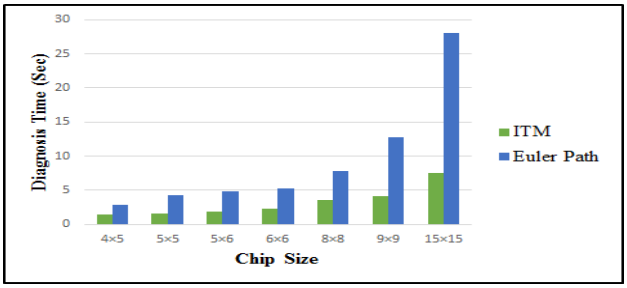

Fig. 7. ITM is compared with Euler path based algorithm [27]

consecutive droplets. Hence $T = (M+N-2)+3(M-1) = 4M+N-5$.

B. Online Testing

It is known that some group of connected cells are unavailable for a DMFB during operation of bioassays. Because these cells may hold droplet for various chemical operations. Thus online testing is desirable to allow system testing with other normal bioassays operations. These group of cells are considered as "obstacle". We assume that the schedule of bioassay operations on a microfluidic biochip is known as *apriori*, e.g., using methods described in [21]. So we can assume that this blocked cells are unvisited cells and add these cells to F. Now, we can pick an element from F which have 3 visited neighbours and apply our method. If any of the cells are not available for this time slot, the test droplet must wait in the current cell until it is available. Total test time for the off-line testing is calculated as earlier for a $M \times N$ biochip. Thus the total incurred test time for on-line testing is equal to the summation of the actual time (T) and the total waiting time.

VIII. Simulation Result

Our proposed algorithm ITM is implemented in C language and executed on intel $i5$ processor having 4 GB RAM. This section compares the test time result obtained using ITM with the method presented in [11], [14], [15], [23], [24]. The comparisons are made based on the two basic parameters: Test Time and Diagnosis Time. Table I shows the comparison of the test time of ITM and the methods presented in [23], [24]. Column 1 represents that the rectangular biochip size varies from 4×5 to 10×10. Column 2 and 3 in Table I represent the test time (TT) of [23] and [24]. Column 4 shows the test time (TT) of our algorithm. Diagnosis time (DT) of ITM is shown in column 5 in CPU time. The percentage of improvement (%Imp) of test time is calculated using Equation 1.

Table I shows that ITM achieves the average reduction of 57.25% and 16.53% respectively compared to those algorithms reported in [23], [24]. The length of a time slot is equal to the droplet transportation time between two adjacent cells, i.e., 62.5 ms when the movement of the droplet is controlled using a 50 V actuation voltage with a switching frequency of 16 Hz. [16]. Hence to diagnose the single fault in average case ITM takes 2.95 sec. It is evident from Table I that our proposed method is much better than the methods presented in [23] and [24]. ITM is also compared with the Parallel Scan Like Test and Euler path in offline with the biochip size varying from 10×10 to 50×50 which is shown in Fig. 6. Fig. 7 shows that the testing and diagnosis time of ITM is much better than the method based on the Euler path. Incurred test time of ITM is compared with the different algorithms which are shown in Table II. Our proposed method can find out multiple defects within very less time which is shown in Fig. 8. Hence, from the results, we can draw this conclusion that our proposed algorithm can test and diagnose multiple faults fairly in less time.

$$\%Imp = \frac{(Other - Our)}{Other} \times 100\% \quad (1)$$

TABLE I
COMPARISON OF TEST TIME BETWEEN ITM AND ALGORITHM [23], [24]

M × N	TT [23]	TT [24]	TT of ITM	DT of ITM	%Imp of TT [23]	%Imp of TT [24]
4 × 5	24	20	16	1.38	33.33	20
5 × 5	31	23	20	1.63	35.48	13.04
5 × 6	40	26	21	1.88	47.5	19.23
6 × 6	50	29	25	2.31	50	13.79
6 × 7	60	32	26	2.5	56.67	18.75
7 × 7	71	35	30	2.81	57.75	14.29
7 × 8	83	38	31	3	62.65	18.42
8 × 8	97	41	35	3.56	63.92	14.63
8 × 9	112	44	36	3.5	67.86	18.18
9 × 9	127	47	40	4.13	68.5	14.89
9 × 10	143	50	41	4.25	71.33	18
10 × 10	161	53	45	4.44	72.05	15.09
Average improvement of testing time: 57.25% [23] and 16.53% [24] **Average time for diagnosis of faulty cell: 2.95 sec [23], [24]**						

TABLE II
COMPARISON WITH THE TEST TIME AMONG THE PROPOSED METHOD ITM, PIPELINED SCAN-LIKE TEST (PISTL), PARALLEL SCAN METHOD (PSL), SCAN PATH-BASED METHOD (SP), VERTICAL STRIP METHOD (VS)

Chip Size	ITM	PISLT [15]	PSL [14]	SP [14]	VS [11]
6 × 6	25	47	86	72	88
11 × 7	46	78	134	154	168
15 × 15	70	138	320	450	510
480 × 640	2555	5587	328800	611400	691360

IX. CONCLUSION

In this work we proposed a heuristic algorithm to minimize the test time for DMFBs with regular shapes. Our algorithm can perform exact fault localization within very less time. As we use multiple droplets for fault diagnosis, our incurred test time is better as compared to the existing algorithms.

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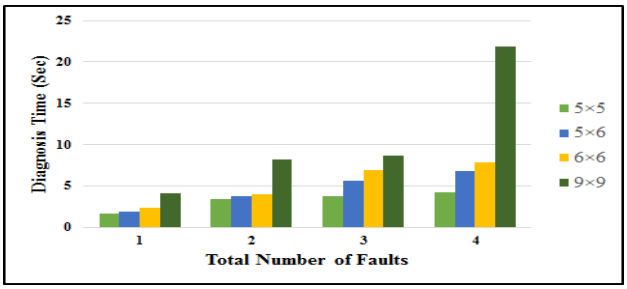

Fig. 8. Multiple Fault Diagnosis Time using ITM

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