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A Droplet Clustering and Residue Removal Technique for Cross-contamination Avoidance in Digital Microfluidic Biochip

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***Abstract*: Progressive research on design automation of digital microfluidic biochip is helping biochips to emerge as an effective alternative for several pathological experiments in the laboratories. This is often deployed for multiplexing several bioassays under optimized space and time constraints. We refer the droplets associated with bioassay of sample or reagents as serviceable droplets. During these assay operations the electrodes often endure multiple utilizations to provide a time optimized routing schedule. Here the residue left by one bio-molecule may contaminate the serviceable droplets used in the subsequent assays. Minimization of such cross-contaminations under the strict adherence of time is an important aspect of research on design automation of digital microfluidic biochip. In this paper, a time optimized routing scheme has been proposed through clustering of routing areas for different serviceable droplets by area analysis. This method helps to reduce both intra and inter sub-problem cross-contamination. In this method, the droplet traces belonging to different sub-problems are partitioned into some regions to produce a contamination minimized routing schedule. This technique effectively keeps track of cross-contamination factors between two successive sub-problems. However the method cannot totally eliminate the possibilities of cross-contamination among multiple sub-problems. Thus residue removal through rinsing of selective electrodes is still required where contamination can't be avoided. Accordingly a time optimized residue removal operation has been proposed for intersecting electrodes through wash droplet scheduling. Experimental study of the proposed technique shows better result over some standard algorithms.**

Keywords: Cross-contamination, digital microfluidic biochip, droplet routing, residue removal, wash droplet scheduling

I. INTRODUCTION

Digital microfluidic biochip (DMFB) is a recent technology that enables proficient on-chip fluid management. This technology is being used in large scale for developing full custom chip also. DMFB is a portable and cost effective device. It has evolved more efficiently over the earlier concept of continuous fluid flow based biochip, which was based on micro-channel fabrication and micro-valve operation for fluid movement [5]. Major drawback of the older version of biochip is the capability of performing bioassays in isolation. Multiple
assay operations were not feasible in continuous fluid flow based technique. DMFB is a revolutionary finding over the earlier continuous fluid flow based technique. In DMFB the operational ability has attained a great extent of reconfigurability and reusability. Multiple assay operations can be performed over DMFB. It is efficient in performing continuous sampling and analysis of real time biochemical assays which is utilized in clinical and pathological diagnosis. Apart from the different complex biomedical procedures like gene sequencing, protein crystallization, drug discovery and cell sorting have been executed on DMFB [8, 10 and 23].

DMFB basically works with micro liter of pico liter droplets. DMFB consists of two dimensional micro-arrays of electrodes as its base. Micro-array of electrode consist different peripheral components like optical detection sites, dispensing ports etc. as shown in the figure 1 [3, 6].

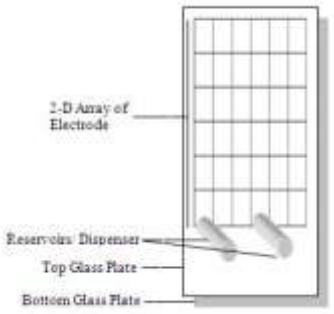

Figure 1. The schematic view of digital microfluidic biochip

The microarray cells of digital microfluidic biochip are made up of two parallel plates and an inside filler medium like silicone oil. The droplet moves through this filler medium. The lower level plate has a patterned array of individually controllable electrodes where as the top plate has a coating of continuous ground electrode as shown in figure 2 [5, 11].

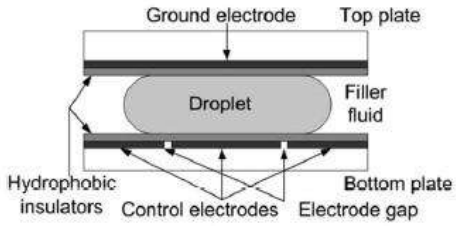

Figure 2. Unit Cell of digital microfluidic based biochip

In DMFB, droplet movements are controlled by a series of activation and deactivation of control pins along the desired path on electrode array. Electrodes are activated and deactivated in sequence through a time varying supply voltage. Electrode activation and deactivation sequence helps to generate an electro-hydrodynamic force which guides the droplets along the desired paths. The phenomenon is known as electro-wetting [1]. However droplets are physically guided along the horizontal or vertical direction or in a combined mode of path switching. Diagonal movement of any droplet is not observed. Droplets are initially dispensed on a cell (unit component of electrode array) from a designated reservoir. It is then transported through a pre-determined path up to a desired cell for mixing or diluting. Multiple droplet movements are always performed along a segregated path. This is to avoid unwanted mixing of two different droplets. In order to maintain this segregation between two different droplet paths, always one unit cell is left blank around the location of a particular droplet. This is known as critical region of a droplet as shown in figure 3 [4, 7].

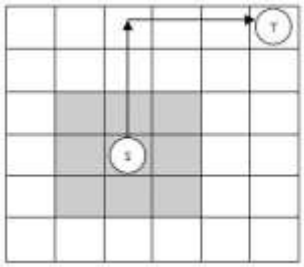

Figure 3. Critical region of droplet marked with S

Efficient controlling of droplet transportation through management of underlying electrodes is a major concern. According to the mode of addressing the electrode cells, DMFB is classified into direct addressing and cross-referencing modes [2]. The direct addressing mode comprises of dedicated control pin for each electrode. In a (N × M) dimensional biochip, total (N.M) number of control pins will be required for direct addressing scheme. Here electrodes are addressed and controlled individually and directly. The control of direct addressing biochip is very flexible. But the concept is not suitable for large sized DMFB performing large number of biological tasks. The manufacturing cost grows rapidly. In order to overcome this issue, a concept of cross-referencing was introduced. This is basically row – column addressing technique of two dimensional micro-arrays. Number of pin requirements for cross referencing biochip is less. In cross referencing biochip we do not use separate addressing for every electrode. Instead of that we use (N + M) number of control pins for (N × M) dimensional chip. But here electrode interference is a major issue and to perform concurrent manipulation of multiple droplets, multiple row and columns of two-dimensional array are activated and deactivated in sequences. Suppose a droplet resides on a particular electrode location (x, y) as shown in figure 4 (a). This electrode needs to be activated by applying high voltage through an external pin. Simultaneously all other diagonal and non-diagonal adjacent electrodes need to be deactivated by connecting to a single pin having a low voltage supply. No other droplet can reside on any of these deactivated electrodes at the same time instant. Successful fluid transportation through the chip area without unnecessary mixing necessitates this electrode activation sequence. Adjacent cell (x´, y´) needs to be activated to transport the droplet to the adjoining location while deactivating the previous position as shown in figure 4 (b) [29]. However this type of control might cause in unintentional activation of extra electrode which may cause irrelevant movement and erroneous mixing of two different droplets.

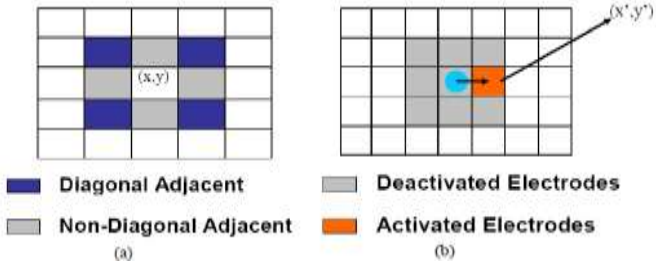

Figure 4. Electrode activation sequence

In recent times various electrode control mechanisms have been proposed to address the issues with growing number of control pins [13, 25 and 27]. The research is simultaneously progressing to address the complicacies of droplet routing in cross referencing biochip [21]. Droplet routing is one of the fundamental issues in design of DMFB. In DMFB, we route a droplet from a source electrode to destination electrode. This is known as a two-pin net as shown in figure 5 (a), where a droplet marked with S is initiated from that specific location and terminates at T. Apart from this, we have a concept of three-pin net. There two separate droplets from different sources (A and B) converges and merges at M and then after mixing the droplet is routed towards a single target T as shown in figure 5 (b) [19].

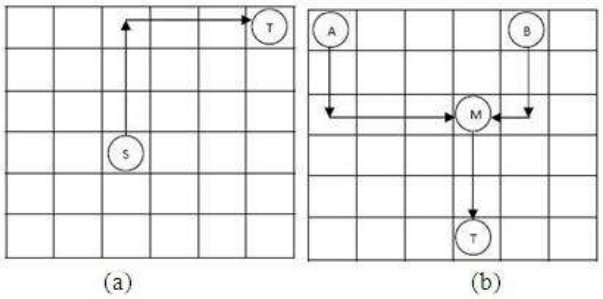

Figure 5. Different types of nets (a) Two pin net and (b) Three pin net

An Initial work of this paper [31] was presented at the IEEE 12th International Conference on Intelligent Systems Design and Applications (ISDA 2012), Kochi, India, November 2012

Droplet routing has gained further importance following to the concept of reconfiguration in DMFB. This reconfiguration causes multiple droplets to share same electrode in different time stamp. This has been noted as a major drawback for different automated droplet routing methods. Cross-contamination among different droplets with such repeated usage of cells is predictable. Cross-contamination occurs when through an inadvertent situation, residue left behind by one droplet transfers to another droplet through some non desirable means [16, 26]. Cross-contamination occurs at intersecting cells, where routing paths of two droplets intersect, and the locations are called 'cross-contamination site'. This will result in ambiguous assay outcome which may also lead to wrong diagnosis.

The cross-contamination problem can be solved either by using wash droplet operations or avoiding intersections during path planning [22]. Wash droplet operation is basically a guided transportation of rinsing liquids from its reservoir through the cross-contamination site to a waste reservoir. Once a droplet passes through the intersecting cell, the wash droplet is scheduled to pass through the cell before arrival of the next droplet. Residue left by initial droplet on the intersecting site will be removed by the transportation of wash droplet through the cross-contamination site. However scheduling of wash droplet along with the routing of serviceable droplets is surely an extra overhead. Complexity of such combined routing solutions is quite high as of the existent methods. Accordingly an efficient routing scheme is required, which will be able to optimize the path planning for multiple serviceable droplets with least possible overlaps and minimum use of washing operations.

In this paper, an initial discussion is made on staircase partitioning approach of different nets followed by efficient routing schemes of serviceable droplets along with wash droplets [31]. This method experimentally established to be an efficient one for intra sub-problem cross-contamination aware routing. Next, another heuristic clustering technique has been proposed for inter sub-problem analysis. The proposed method attempts to minimize the intersecting paths by area analysis of serviceable droplets. This method has given excellent contamination aware routing solutions through contamination aware inter sub-problem analysis. The findings are better than some of the recently proposed methods. The effectiveness and scalability of the newly proposed technique has been experimentally established over different bioassays. It has been found that contamination sites are quite less in numbers in compare to other methods. In order to clean the remaining contamination sites; an integrated washing operation with droplet routing has been proposed.

The rest of the paper is organized as follows; Section II provides a background of DMFB related prior work on droplet routing and cross-contamination management. Section III describes problem formulation. Staircase partitioning approach for cross-contamination management in intra sub-problem analysis has been discussed in section IV. Section V illustrates an advanced droplet clustering technique for contamination aware routing with optimized wash droplet scheduling method. Section VI establishes the efficiency through experimental findings along with its comparative analysis with prevalent techniques. Finally, conclusions are drawn in section VII.

II. Background and Some Existing Works

Emerging capabilities of DMFB has motivated many researchers to work for more customizable designs. In literature there are many references on droplet routing in DMFB based on different methods. A progressive Integer Linear Programming (ILP) based algorithm has been proposed in [12]. At first they count a time to travel from source to sink and then an iterative ILP solver is used to solve routing within a stipulated time benchmark. In [9] a network flow based two stage method has been proposed. The authors prepares an undirected graph considering the source and destination pair and then perform a high level routing based on "Minimum Cost and Maximum Flow" technique. But the routing is not tuned because the dependency graph was prepared by only considering the fluidic constraint. So final scheduling is not possible through high level routing. A bypassibility and concession based approach was proposed in [11]. Here bypassibility is a measure of probability for an unrouted droplet being routed by bypassing the blockages and concession mechanism manages deadlock conflict between two droplets. Concession mechanism works by assigning priority on droplets depending on their distance from the target. But this paper couldn't manage the timing factors efficiently. A clique partitioning based method on cell categorization by grouping multiple droplets has been proposed in [15]. But the method is inefficient in terms of considering electrode interference and time constraints. A fault tolerant routing mechanism was proposed in [14]. In a pre-synthesis analysis the authors have studied the life span of each electrode and then by discarding the fault prone electrodes the authors have proposed a routing plan for droplets. However another article [18] talks about a global moving vector analysis for construction of a convincing routing path to minimize the number of electrodes. Especially they proposed an entropy based mechanism to schedule the nets and then a compaction technique using dynamic programming to minimize the latest arrival time. A concept of cross-router was proposed in [24]. The authors have used a weighted maze routing framework to move an amount of droplets together to optimize the parallelism in minimum time. Thus it claimed to reduce the total electrode requirement; hence the possibility of fault due to mechanical breakdown in electrode can be minimized. A Soukup's routing algorithm based concurrent path allocation method to multiple droplets was proposed in [19] along with stalling and detouring conflicting droplets. The authors have resolved the conflicts based on their manhattans to target. Another concurrent path allocation method based on classical shortest path algorithm for two-pin net and three-pin net was proposed in [21]. In a few comparatively recent works, authors of [28] and [32] have explored the routing challenges with the help of multi objective optimization methods for both two-pin and three-pin nets. In [28], an ant colony optimization approach was applied to optimize the number of electrode usage and routing completion times. Basically they have proposed a two tier solution for optimized path planning. In the first phase a guided ant movement is observed for formation of bounding box between each source and target pair and then a final scheduling is performed to generate optimized route for each droplet. In [32] a particle swarm optimization based approach for droplet routing has been proposed. The process

mainly operates in two phases where initially authors perform clustering of state space and classification of nets into designated clusters. This helps them to reduce solution space by redefining local sub optimal target in the interleaved space between source and global target of a net. In the next phase they resolve the concurrent routing issues of every sub optimal situation to generate final routing schedule.

All these algorithms don't apply any restriction on choice of electrodes to route any serviceable droplet. To avail optimum result by minimum time and minimum electrode usage, these algorithms often use same electrodes at different time instances for different droplet. This may lead to contamination of one droplet due to unwanted mixing with residue left by previous droplet. In literature we have studied some considerable amount of existing work. In [17] a contamination aware droplet routing algorithm for DMFB has been proposed. In order to reduce the routing complexities and the used cells, authors first construct preferred routing tracks by analyzing the global moving vector of serviceable droplets to guide the droplet routing. To cope with contaminations within one sub-problem, they first applied a k-shortest path routing technique to minimize the contaminated spots. Then, to take advantage of multiple wash droplets, a minimum cost circulation algorithm (MCC) has been adopted for optimal wash-droplet routing to simultaneously minimize used cells and the cleaning time. Furthermore, a look-ahead prediction technique has been used to determine the contaminations between successive sub-problems. After that, authors suggested to simultaneously clean both contaminations within one sub-problem and those between successive sub-problems by using the MCC-based algorithm to reduce the execution time and the used cells. A wash droplet management technique to clean the contaminations on the surface of the microfluidic array has been proposed in [22]. But scheduling of wash droplet does not restrict the extra used cells and execution time of bioassay. This results in degradation of the reliability and fault-tolerance issues. Here authors first propose to construct a preferred routing track by analyzing the global moving vector of droplets to reduce the routing complexity and the used cells. In order to manage the contaminations within one sub-problem, they have applied a *k* -shortest path routing technique to minimize the contaminated spots. Then, to take advantage of multiple wash droplets, minimum cost circulation (MCC) algorithm was used. The method claimed to provide optimal wash-droplet routing and simultaneous minimization of used cells and the cleaning time. They have also used a look-ahead prediction technique to determine the contaminations between successive sub-problems. In [16, 30] author propose a droplet-routing method that avoids cross-contamination in the optimization of droplet flow paths by disjoint droplet routing and synchronization of wash-droplet routing with serviceable droplet routing. In order to reduce the duration of droplet routing while avoiding the cross-contamination between different droplet routes, an optimization technique has been used to minimize the number of wash operations that must be used between successive routing steps. Proposed method has been experimented over two real-life biochemical applications for evaluation and analysis. Another wash-operation synchronization method to manipulate wash droplets to clean the residue that is left behind by sample and reagent droplets has been proposed in [20]. They have synchronized wash-droplet routing with sample/reagent droplet-routing steps by controlling the arrival order of droplets at cross-contamination sites. This technique minimizes droplet-routing time without cross-contamination, and it is especially effective for tight chip-area constraints.

III. PROBLEM FORMULATION

A. Droplet Routing under Different Fluidic Constraints

Bioassay protocols generated from architectural level synthesis [2] are used in placement phase of geometry level synthesis to place modules on a 2*D* biochip array [4]. Droplet routing is performed after module placement, where the module locations and I/O ports are used as inputs to the routing problem. Each routing path is generally considered as 2-pin or 3-pin net. Major challenge during droplet routing is to route the droplets successfully from their source to the target locations without any timing conflict or intersection or overlap in routing path of different nets to avoid contamination effect. In this paper two different solutions have been discussed on intersection minimized droplet routing for DMFB within optimized routing completion time.

Routing time of a given net is the time or clock cycle required for the droplet belonging to the source location to reach the corresponding target location; this is also called arrival time (*T*). As concurrent droplet routing is performed for efficient resource utilization, a term called latest arrival time (La. time) [11] is calculated, which is the maximum of the routing time/arrival time of all the nets. During droplet routing two major fluidic properties have to be observed always. Let d_i at location (x^t_i, y^t_i) and d_j location at (x^t_j, y^t_j) denote two independent droplets at time *t*. Then, the following constraints, generally called *Fluidic Constraint*, should be satisfied over every timestamp *t* during routing;

- Static Fluidic Constraint: $|x^t_i - x^t_j| > 1$ or $|y^t_i - y^t_j| > 1$
- Dynamic Fluidic Constraint: $|x^{t+1}_i - x^t_j| > 1$ or $|y^{t+1}_i - y^t_j| > 1$ or $|x^t_i - x^{t+1}_j| > 1$ or $|y^t_i - y^{t+1}_j| > 1$

B. Scope of the Work

A 2*D* biochip array can be conceptualized as a rectangular grid graph *G* (*V, E*) having *V* as the nodes of the graph, complying with the electrodes on the chip and *E* corresponds to the rectilinear edges connecting the vertices. Given a set of *n* nets $N_1, N_2, \ldots, N_n$ with their source-target locations N_i (S_{Ni}, T_{Ni}) specified over the grid graph, our aim is to find a concurrent routing scheme so that it will comply with the following issues,

a) Avoid any overlap or intersection between two nets Ni and Nj ($\Rightarrow$ Ni(V) $\cap$ Nj(V) is minimized or = Φ) where N(V) denoted the set of electrodes constituting the path for net N

b) Minimize routing completion time or Latest arrival time among all the nets ($\Rightarrow$ min (T_{MAX})) where (T_{MAX} = [**MAX** {$N_1(T_1)$, $N_2(T_2)$, . . . , $N_N(T_N)$}]) and N_1 to N_N represent the complete net list of a given sub-problem and N(T) denotes that time T is elapsed to complete the routing of net N

c) Schedule wash operations for any intersection region between two different nets. In case of wash operation scheduling, additional time will be required for routing and rinsing through wash droplets. Accordingly, T_{MAX} may increase in a few situations. Let T_W is the additional overhead on T_{MAX} due to washing. Hence the modified objective function will be [**min (T_{MAX} + T_W)**].

For the ease of routing, and to reduce computational complexity, all the bioassay protocols used as input to the problem are decomposed into smaller sub-problems, and experiment is performed over them. Cross-contamination can occur among any pair of droplets belonging to a particular sub-problem which herein after will be referred as intra sub-problem cross-contamination. Similarly any droplet belonging to a particular sub-problem can contaminate another droplet of a subsequent sub-problem. This might happen due to sharing of same electrode between such pair of droplets and cross-contamination among pair of droplets from two consecutive sub-problems is referred here as inter sub-problem cross-contamination. Thus some techniques have to be developed to prevent such unwanted mixing of multiple droplets during transportation. In the next section, staircase partitioning and washing approach [31] for cross-contamination avoidance in droplet routing is discussed with an example layout diagram.

IV. Staircase Partitioning of Routing Area for Intra Sub-problem Cross-contamination Minimization

Basic objective of this method is to minimize the electrode sharing among different serviceable droplets of a same sub-problem. The proposed solution for cross contamination avoidance is primarily designed into two phases. In the first stage each of the given sub-problems has been analyzed with respect to total number of serviceable droplets and their corresponding source-target locations. This analysis was further applied to perform a modified staircase partitioning of the two dimensional board. The optimized objective of this partitioning is to logically separate the board into couple of zones. These zones will contain less number of serviceable droplets. More specifically the partitions were performed by allowing maximum two nets for a same zone. Logically this has the probability of reducing the chances of interference. It is quite simple to route two different serviceable droplets if they belong to a non-overlapping zone. But experimentally it has been found that such ideal partitioning is not always feasible. Here it has been found that source and target of a corresponding serviceable droplet might get split in to two different zones. In such cases optimized path can be tuned by an efficient routing management strategy.

This partition trace initiates from left uppermost source of droplet. Trace proceeds along the vertical edge of a logical bounding box between source-target electrode cell pair. This trace flips on finding any horizontal cross-section of own or any other bounding edges. A particular trace stops on meeting any other source or target location. This trace marks out two different zones. The tracing is repeated if any zone contains more than two nets. The method is further explained with Figure 6. In 6 (a), all serviceable droplets have been depicted with their source and target locations. Accordingly we can see that there are five nets and their source locations are marked with S1 to S5 and respective target locations by T1 to T5. Bounding box between source and target locations of every net is shown in figure 6 (b). These bounding boxes are marked with dashed rectangular shaped areas. Routing area partitioning has been shown in figure 6 (c). Here to partition the board based on the location of nets; method first checks the column 1 of the microarray. As soon as it founds a vertical edge of a bounding box (from S2), it starts tracing along that edge. Once it detects another droplet location (i.e. a target at T2) it stops there. The droplets (S2 and T2) located on the line of this trace and on the left hand side of the trace will belong to left sub zone. Accordingly the rest of the electrodes of the right of this partition will remain in the right sub zone. The partition traced out from the column 1 of a board is considered to be the first sub-division of the whole board. Hence in its equivalent tree like representation in figure 6 (d), this will form the respective left and right child of the root. In level 1 node of the tree, we see that left child is containing only a single source – target pair (i.e. S2 – T2) but the right child has multiple nets. Hence further partitioning is required for the right sub zone. Accordingly it proceeds with the partition of right sub-tree in same manner. Finally this process will end when all the leaf nodes will contain maximum two or lesser number of nets or a part of the net.

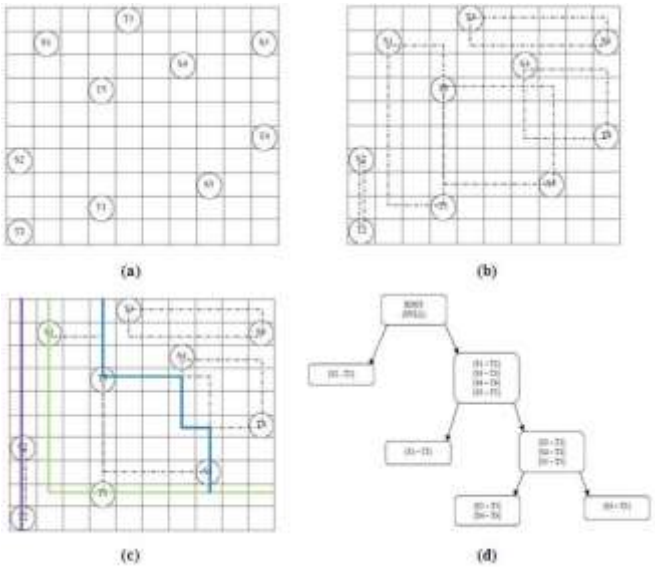

Figure 6. Staircase partitioning approach (a) Location of source and target electrodes for five nets (b) Marking of bounding box for all nets (c) Staircase partitioning of two-dimensional microarray in to multiple regions (d) Tree structure representation of partition

Thus at initial stage this staircase partitioning of the routing area for a specific sub-problem creates a set of routing zones. This staircase partitioning approach is summarized by a pseudo code in Algorithm 1.

Algorithm 1: Staircase partitioning of routing regions of different nets

Input: Grid dimension (n × m) and List of nets, N_k $[(x_s, y_s); (x_t, y_t)]$ where ($1 \leq k \leq n$) of any sub-problem in terms of respective source (x_s , y_s) and target (x_t, y_t)

Output: Partitioned routing regions

begin

$S^k [\,][\,] \leftarrow (x_t, y_t)$ of all nets;

```
    T^k [ ] [ ] ← (x_t, y_t) of all nets;
    for k ← 1 to n do
        w ← x_s ~ x_t; h ← y_s ~ y_t;
        x_s´ ← x_s ± w and x_t´ ← x_t ± w;
        y_s´ ← y_s ± h and y_t´ ← y_t ± h;
        //opposite corners of bounding box
        S´ ← (x_s´, y_s´); T´ ← (x_t´, y_t´);
    end
    for j ← 1 to m do
        for i ← 1 to n do
            if ([i][j] in S [][] = True)
            ∂ [i][j] ← begin partition;
            j^temp ← j; i^temp ← i;
            while (λ [] [] not found) do
                i^temp ++;
            if ([i^temp][j^temp] in S´ = True)
            while (λ [][] not found) do
                    j^temp ++;
                    if ([i^temp][j^temp] in T [][] = True)
                    λ [i][j] ← [i^temp][j^temp];
                    if ([i^temp][j^temp] in T´ = True)
                      break;
                    end
            if ([i^temp][j^temp] in T [][] = True)
            λ [i][j] ← [i^temp][j^temp];
            end
        end
    end
end
```

In algorithm 1, S^k and T^k array are used to keep track of respective source and target coordinates of all nets. S´ and T´ are used to keep track of extended corners of all k number of bounding boxes. ∂ [][] is marking the starting coordinate of each partition trace and λ [][] is holding the terminating coordinate of the trace. So $\partial^k\lambda^k$ holds the initial and terminating coordinates of k^{th} partition. Thus a sub-problem is partitioned into a few routing zones to facilitate cross-contamination minimized routing of serviceable droplets. The method is based on combination of horizontal and vertical tracing through bounded box boundaries of associated nets.

In the next phase path planning for all serviceable droplet is performed. Here majority of the droplets are traced within their designated partition to assign a path for its target. But if the target location occurs in some different partition then path tracing is modified to avoid intersection with the droplets belonging to that partition. This guided routing doesn't ensure complete avoidance of intersection. Such contaminations are avoided by optimized wash droplet scheduling. During path tracing all possible intersections are tracked. Then the routing plan is modified to accommodate wash droplet operation along with serviceable droplets. Wash droplet is scheduled after arrival of the first droplet at the intersecting point to perform wash. There after the second droplet is scheduled to pass on through that site. A wash droplet operation has been depicted in figure 7. Here partitions of routing areas for different droplets are shown by blue and green colored lines, and the contamination site is marked by gray color in figure 7 (a). Droplet from the source S1 reaches the contamination site first and moved towards the target location T1 without any difficulty. Now, before moving the droplet from source S2 through the contamination site, washing need to be performed. Accordingly wash droplet is dispensed into the chip from a wash reservoir marked by green box. It is then routed through the contamination site marked in grey for rinsing and then it is transported to the waste reservoir, which is marked by orange box in figure 7.

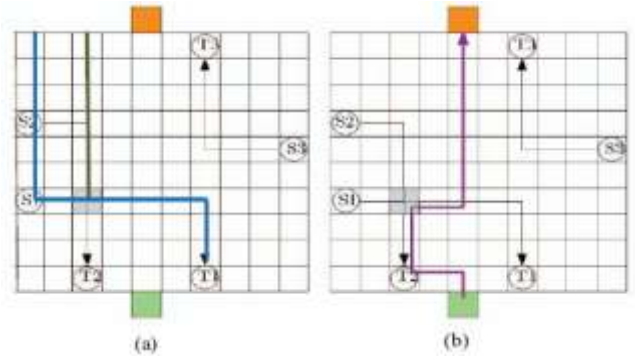

Figure 7. Example of wash operation (a) Locating contamination site (b) Wash operation and complete routing

Let a serviceable droplet from S1 reaches the contamination site at time cycle t^n following to this a wash droplet can perform residue removal earliest by time cycle t^{n+3}. During this time, the droplet from S2 is stalled till the wash operation is completed and earliest by t^{n+5} it reaches the contamination site. Thereafter it moves towards the corresponding target location T2. The routing path for the wash droplet is shown by the pink colored line in figure 7 (b). The droplet from S3 to T3 is not hampered by the washing operation of the two nets $S1-T1$ and $S2-T2$ and is routed concurrently. This method was applied up on a few basic test benches. Experimental study of this method in section VI establishes its efficiency over some existing methods.

When two consecutive sub-problems are analyzed together to perform contamination aware routing for inter sub-problem analysis, sometimes it returns ambiguous results on partitioning the routing regions. This limitation has been mostly observed for cumulatively large number of nets and if they are partially overlapped either by source or by target or in both. In order to address this limitation, a new intrusive droplet clustering method based on area analysis of overlapping or non-overlapping bounding boxes is illustrated in the next section.

V. Droplet Clustering and Wash Droplet Scheduling for Inter Sub-problem Cross-contamination Minimization

Cross-contamination minimization is now an essential requirement for droplet routing. Single biochip may be utilized repeatedly to perform consecutive on-chip assay operations. So possibilities of contamination among various serviceable droplets are not only limited within a particular sub-problem. There are equal possibilities that droplet of a particular sub-problem may be contaminated through the residues left by droplets of preceding sub-problem. Hence cross-contamination aware path planning of droplet routing should also consider the droplet paths of two consecutive sub-problems. Accordingly, the modified objective function for contamination aware path planning of droplet routing over two-dimensional electrode array can be explained as;

a) $[(N_i^S \cap N_j^S) \cup (N_i^{S´} \cap N_j^{S´})] = \Phi$, where N_i & N_j denotes i^{th} & j^{th} droplets of same sub-problem and N^S & $N^{S´}$ respectively denotes nets of two consecutive sub-problem S & S´

Other two objectives will remain same as discussed in the previous section.

A. Droplet Clustering Method

An assay is considered as a sub-problem and every sub-problem (S) contains multiple nets (N). Our objective is to generate an optimum sequence for assay operation so that two consecutive assays will have the least possible intersections or no intersection. In order to generate such sequence, we propose to analyze the assays in pair to generate a sequence of independent pairs. Independent pairs use to have least possible cross-contamination sites among themselves. Generation of independent pairs of nets are guided by the following three phases.

a) ***Integrated Clustering of droplets belonging to a sub-problem pair*** – A set of sub-problems are considered in all possible pairs for this analysis. During each simulation cycle any such pair is analyzed to determine their mutual dependencies. Accordingly, bounding region between source-target pair for every net is constructed. Then all pairs of nets are compared to determine the number of mutually overlapped electrodes among their bounding regions. This helps to analyze the probability (ρ) of cross-contamination between any two individual nets. We analyze through the area on chip (α) covered by their respective bounded regions and mutual overlapping (ψ) (common guarded region of electrodes) between them. A threshold parameter (μ) has also been set. This threshold factor helps in determining the probability of cross-contamination. During analysis, the following situations may arise;

1. **ρ = 0** if $[\alpha (N_k) \cap \alpha (N_j)] = \Phi$, where Φ signifies no overlapping and N_k and N_j are consecutively scheduled nets of a sub-problem and α(N) denotes the bounded area of net N
2. **ρ = 0** if $[\alpha (N_k) \cap \alpha (N_j)] = \psi$ where $\psi < \mu$
3. **ρ = 0** if $[\alpha (N_k) \cap \alpha (N_j)] = \psi$ where $\psi > \alpha (N_k)$ or $\psi > \alpha (N_j)$
4. **ρ = 1** iff $[\alpha (N_k) \cap \alpha (N_j)] = \psi$ where $\psi \geq \mu$ and $[\psi < \alpha (N_k)$ or $\psi < \alpha (N_j)]$

$$[\mu = ½ [MAX (\alpha (N_k), \alpha (N_j))].]$$

These four conditions summarize that the probability of cross-contamination is high in the last situation only where $\psi \geq \mu$ but ψ is not as large as α for any of the nets.
In this comparison, if a pair of nets has low or zero probability of intersection, then they are grouped in a same cluster and if a pair analysis reveals high probability of intersection then they are partitioned into different clusters.

These four conditions are further explained through figure 8. Here we consider two different droplets, and the rectangular bounding box for the source-target pair of each of the droplet is marked in red and green. In figure 8 (a) two different bounded regions are completely non-overlapping, hence the probability of having intersection among there routing path is nil. Figure 8 (b) depicts that red and green zones are having minimum overlap, which is even lesser than the threshold μ. It is also evident from the figure that both the nets are routable without any intersection. Figure 8 (c) shows that bounded region of green net is completely lying within the red bounded region. Here green droplet can be routed without intersecting the routing path of red droplet. In figure 8 (d) red and green bounded regions are having high mutual overlap, which is higher than the threshold μ. In this situation it is difficult to route them without intersection. As we see that mutual overlapping is very high in 8 (d), so the nets will be partitioned in two different clusters and in rest of three situations, the nets can be kept in same cluster.

This partitioning approach is used to find out some independent and non-overlapping region for droplet transportation. Nets belonging to same cluster can be routed without any cross-contamination but attention should be taken for net belonging to different cluster.

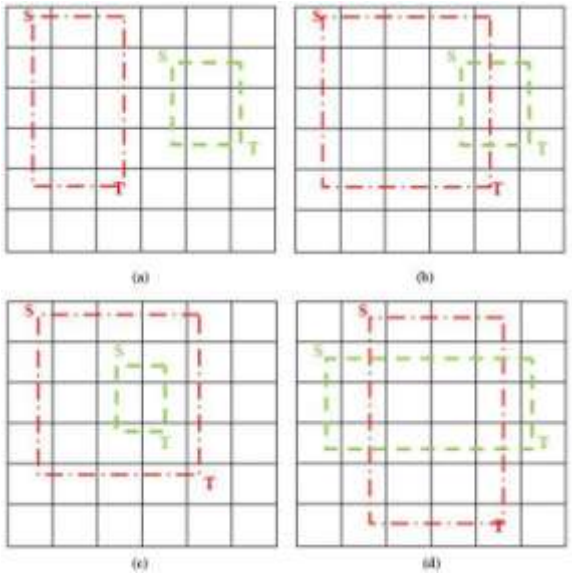

Figure 8. Different overlapping conditions (a) Bounded region of two nets are non-overlapping (b) Bounded region of two nets are least overlapping (c) Bounded region of one net is totally within the bounded region of other net (d) Bounded regions of two nets having maximum mutual overlap

b) ***Generation of intersection minimized routing trace*** - Droplets falling under same cluster are supposed to have non-intersecting routing paths, and they are routed in concurrent manner for efficient routing time utilization. At first, concurrent routing traces are made for all the droplets belonging to the cluster having maximum droplets. Thereafter other clusters are addressed sequentially to generate the routing paths for the droplets lying within it. If any droplet path finds an overlapped electrode then a flip operation is applied to overcome such intersection. This concept of path flip is explained in figure 9.

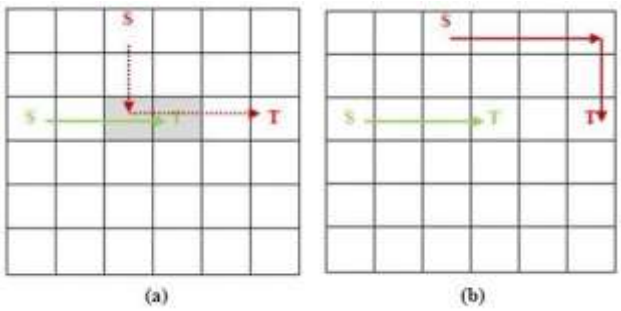

Figure 9. Flip operation on routing path (a) Routing path having intersections (b) Intersection avoidance by route flip

In figure 9 (a), possible routing traces of red and green droplet has been shown. But there two shadowed electrodes are clearly indicating the possibilities of intersecting electrodes. In order to avoid this inadvertent situation, the routing path of red droplet has been flipped to alternative direction by keeping the number electrode requirement same as the previous routing path to complete the routing. This is the basic concept of flip where alternative route may be availed upon availability of free electrodes to avoid undesired intersection or overlapping between two different routing paths.

However this method can't generate cross-contamination free routing traces for all nets. Accordingly the possible contamination points are recorded during path tracing. Thereafter, the routing traces are readjusted for the nets having cross-contaminations to accommodate wash droplet operation.

c) ***Isolated routing*** **–** Once all the traces are finalized, then all serviceable droplets belonging to preceding sub-problem are concurrently routed along the traced path. On completion of the preceding sub-problem its consecutive sub-problem is routed. This isolated routing can be accompanied by required washing operations to remove residues from cross-contaminated sites.

The method for computing probability of intersection is summarized by a pseudo code in Algorithm 2;

Algorithm 2: Droplet partitioning to compute probability of intersection between all pair of nets

Input: Grid dimension (n × m) and List of nets, N_k *[(x_s, y_s); (x_t, y_t)]* where ($1 \leq k \leq n$) of any sub-problem in terms of respective source *(x_s , y_s)* and target *(x_t, y_t)*

Output: Probability of intersection between a pair of nets

```
begin
    S^k [ ] [ ] ← (x_t, y_t) of all nets;
    T^k [ ] [ ] ← (x_t, y_t) of all nets;
    for k ← 1 to n do
        Construct bounded box of k^th net;
        E^k[] ← electrodes within bounded region of k^th net;
    end
    for i ← 1 to n do
        s ← count (E^i[]); t ← count (E^(i+1)[]);
        μ ← [max (s, t)] / 2;
        q ← 0;
        for i ← 1 to s do
            for j ← 1 to t do
                if (E^i[i] = E^(i+1)[j]) {q++;}
            end
        end
        compare (q, μ);
    end
end
```

In the above mentioned algorithm 2, S^k and T^k array are used to keep track of respective source and target coordinates of all nets. E^k[] keeps track of individual electrode numbers lying under bounded region of net k. q is an actual overlapping quotient between bounded electrode array of two consecutive nets, namely E^i[] and E^{i+1}[]. Finally this quotient, q is compared with dynamic threshold parameter μ to decided probability of mutual cross-contamination between two successive pair of nets.

B. Wash Droplet Scheduling

Droplet clustering approach reveals intersection among routing paths in a same cluster, but fails to detect any common routing trace, or intersecting regions between different nets belonging to different clusters. Certain dependencies and complexities among different assay operations may also act as a bottleneck for complete removal of cross-contamination through droplet clustering method. Thus residue removal operation or washing operation will be required to address possible intersections. Accordingly two different washing approaches may be taken for residue removal.

a) Intersection oriented wash droplet scheduling
b) Invasive wash droplet scheduling

Intersection oriented wash droplet scheduling - In the first method, wash droplet is routed from the wash droplet reservoir to cross-contamination site and from the cross-contamination site to waste reservoir. This wash droplet is routed by maintaining the static and fluidic constraints of droplet routing mentioned in section III. In this method, wash droplet is routed in synchronization with two serviceable droplets. The arrival time of wash droplet at the cross-contamination site is adjusted with arrival order of two serviceable droplets. Once the first serviceable droplet approaches towards the contamination site, then the wash droplet is concurrently routed towards the contamination site. But the arrival of wash droplet is stalled unless the first serviceable droplet crosses the cross-contamination point. It is synchronized in such a way that once the first serviceable droplet passes the cross-contamination site, the wash droplet is routed through that electrode. Routing of a wash droplet normally removes the residue left by first serviceable droplet. After residue removal by wash droplet routing, the second serviceable droplet is permitted for routing through that electrode. This method is explained in figure 7. This method will follow the following steps mentioned in figure 10 to properly remove the residues.

Step 1: Detect the cross-contamination probable droplets from inter sub-problem analysis

Step 2: Trace out projected routing paths for all such droplet to determine their routing orders

Step 3: Schedule the arrival order of wash droplet and two serviceable droplets

Step 4: Finally tune the routing –timing of all serviceable droplets along with the required number of wash droplets

Figure 10. Procedure for intersection oriented wash droplet scheduling

Invasive wash droplet scheduling - The invasive wash droplet scheduling method determines wash droplet routing path to emancipate the movement of serviceable droplet having the projected probability of contaminating its subsequent droplets between different clusters. In this phase, before initiation of the serviceable droplet routing, required number of wash droplets (ideally the number is same with the number of serviceable droplets being analyzed to be routed in a single cluster) are dispensed from the wash droplet reservoir and those are routed towards the source coordinates of each such serviceable droplet. Each of the wash droplets is kept at a minimal critical distance of serviceable droplets in such a manner so that it can follow the route of serviceable droplet by maintaining the desired fluidic constraint. Here we assume that a single wash droplet is able to absorb the residue left by each serviceable droplet at multiple locations. Though this method of invasive wash operation consumes excess time added to the routing time, however it assures contamination free routing of droplets belonging to different clusters. This invasive procedure is summarized in figure 11.

Step 1: Determine the serviceable droplets having probability of cross-contamination

Step 2: Route wash droplets from reservoir to a nearby location of cross-contamination prone serviceable droplets

Step 3: Schedule wash droplet movement to ensure proper and timely rinsing

Step 4: Finally tune the routing trace of all serviceable droplets along with the required number of wash droplets

Figure 11. Procedure for invasive wash droplet scheduling

VI. EXPERIMENTAL ANALYSIS

The staircase partitioning algorithm (Section IV) and the proposed extended droplet clustering algorithm (Section V) were implemented in C along with different washing operations (Section IV and V) on a PC running on Intel chip with 2 GB RAM and 2.5 GHz clock speed in Linux platform.

A series of simulation based experiments were performed on different bioassays of [16, 22]. These bioassays represent two biological processes, in-vitro diagnostics and protein reactions respectively. Details of these suits are explained in Table I. In table I, ´Name´ represents the name of different bioassays, ´Dimension´ denotes the area of microfluidic array in use, ´#SP´ denotes number of sub-problem under a test suite, ´#Net´ represents number of nets and ´D_{max}´ represents maximum number of droplets present under a sub-problem. The main objective is to derive an optimally scheduled droplet routing for achieving high throughput.

Name	Dimension	#SP	#Net	D_{max}
in-vitro_1	16 × 16	11	28	5
in-vitro_2	14 × 14	15	35	6
protein_1	21 × 21	64	181	6
protein_2	13 × 13	78	178	6

Table I. Details of Test Benches Used

In the first experiment, staircase partitioning based contamination aware droplet routing algorithm was simulated for intra sub-problem cross-contamination aware routing. Experimental outcome is recorded in Table II. In table II, #CCS stands for number of cross-contaminated sites, #E means total number of electrodes required to complete routing of a specific bioassay, LaTime and $LaTime^W$ respectively designate latest arrival time without wash operation and with washing operation and CPU utilization during droplet routing with wash droplet scheduling is given through CPU^W. Here latest arrival time is the final routing completion time of a specific bioassay. LaTime and $LaTime^W$ are calculated in terms of number of time cycles elapsed since routing initiation. CPU execution time is given in seconds.

Test	#CCS	#E	LaTime	$LaTime^W$	CPU^W
in-vitro_1	2	246	180	195	0.025
in-vitro_2	0	174	168	168	0.024
protein_1	10	1895	1159	1288	0.214
protein_2	9	3824	1048	1074	0.133

Table II. Experimental results of Section IV on Table I

A summarized result has been recorded in table II, whereas a detailed analysis through the first experiment has been shown in table III. Detailed analysis on all the eleven sub-problems under *in-vitro_1* has been shown where number of partitions, number of intersections, routing completion time without wash operation and with wash operation and CPU execution time has been discussed. Apart from these, similar details for analysis on two hard test benches have been given in the table III. These results were further compared with [16] and the comparison graphs are given in Figure 12, 13 and 14. In these graphs SP_i denotes sub-problem of *in-vitro_1.i*. In table III, #SP, #N, #P, #C, TR_N, TR_W and CPU respectively denote number of sub-problems, number of nets, number of partitions made during analysis, number of cross-contamination sites, normal routing completion time without wash operation, routing completion time with wash operation and CPU execution time without wash operation. Routing time is measured in terms of number of time cycle elapsed since start of the routing and CPU execution time is noted in seconds.

#S	#N	#P	#C	TR_N	TR_W	CPU
in-vitro_l.1	2	2	0	19	19	0.0019
in-vitro_l.2	2	2	0	12	12	0.0019
in-vitro_l.3	3	2	0	17	17	0.0019
in-vitro_l.4	1	1	0	5	5	0.0019
in-vitro_l.5	6	4	1	17	17	0.0021
in-vitro_l.6	4	4	1	10	16	0.0020
in-vitro_l.7	2	2	0	10	10	0.0019
in-vitro_l.8	2	2	0	9	9	0.0019
in-vitro_l.9	2	2	0	10	10	0.0019
in-vitro_l.10	1	1	0	9	9	0.0019
in-vitro_l.11	1	1	0	10	10	0.0019
test_12_12_1 [1]	12	6	7	23	29	0.0182
test_16_16_1 [1]	16	8	13	30	41	0.0223

Table III. Experimental results of Algorithm 1 on *in-vitro_1* Test benches

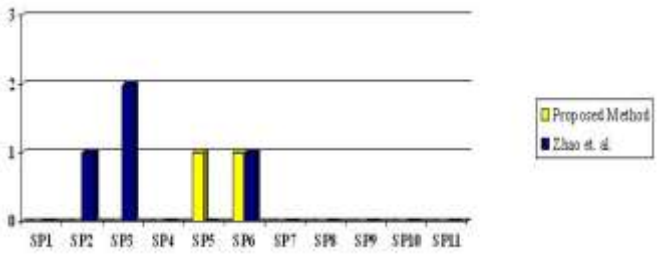

Figure 12. Comparison between Proposed method and [16] on #C

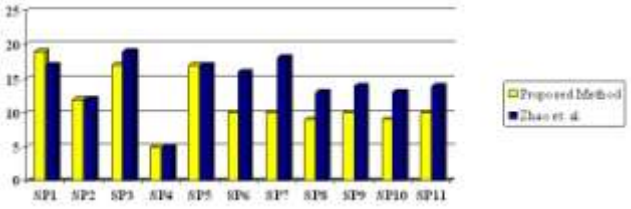

Figure 13. Comparison between Proposed method and [16] on TR_N

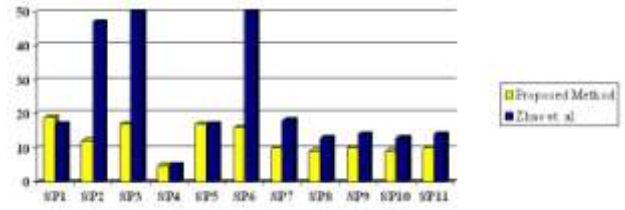

Figure 14. Comparison between Proposed method and [16] on TR_W

Figure 12 shows the improvement in number of contaminating sites in our proposed method. Especially for *in-vitro_1* benchmarks we have noticed the intersecting electrodes (cross-contamination site) are very less in compared to that in [16]. Figure 13 manifests the betterment in routing time before wash operation compared to that in [16], and an implausible improvement is noticed in routing completion time with washing operation (figure 14). Washing operation involves a wash droplet movement alongside the routing of nets through the contamination sites, and charts in figure 14 show the comparative analysis between our proposed method and the algorithm in [16]. We have also simulated our algorithm with a few hard test sets to test its tolerance in a complex bio assay. It is observed from the experimental results that this staircase partitioning method (section IV) followed by a contamination aware routing is working excellently. Also the results upon the hard test sets are quite encouraging.

In the second experiment, clustering based contamination aware droplet routing algorithm was simulated. This can address both intra and inter sub-problem analysis. Here washing operations have been scheduled according to two different methods as mentioned in section V. The summarized result is given in table IV. In this experiment, latest arrival time has been recorded thrice; initially it was computed without wash operation (LaTime), then it was recorded in $LaTime^{WCC}$ for routing with residue removal through intersection oriented wash droplet scheduling and finally in $LaTime^{WINV}$ for routing with wash droplet scheduling through invasive method. #CCS and #E have similar meaning as of table II.

Test	#CCS	#E	LaTime	$LaTime^{WCC}$	$LaTime^{WINV}$
in-vitro_1	1	252	174	182	191
in-vitro_2	0	197	168	168	168
protein_1	3	1933	1239	1257	1324
protein_2	2	3977	1048	1057	1063

Table IV. Experimental results of Section V on Table I

An observation on table II and IV reveals that clustering based routing approach is more efficient than staircase partitioning method. During simulations we have observed that staircase partitioning strategy is yielding more intersecting sites than the later.

We also observed that the efficiency of droplet clustering based contamination aware routing algorithm is better than the results of [16, 22]. These three methods have been compared for number of contamination sites, number of electrode usage to complete routing and latest arrival time without washing. The results are respectively shown in table V, VI and VII along with a comparative graphical representation in figure 15, 16 and 17. These three comparisons establish that the proposed method is drastically reducing the cross-contamination sites. More significantly total electrode usage and latest arrival time is also better.

Test	#CCS		
	Proposed	[22]	[16]
in-vitro_1	1	21	4
in-vitro_2	0	5	0
protein_1	3	82	18
protein_2	2	61	11

Table V. Comparison of Proposed Method with [16] and [22] for #CCS

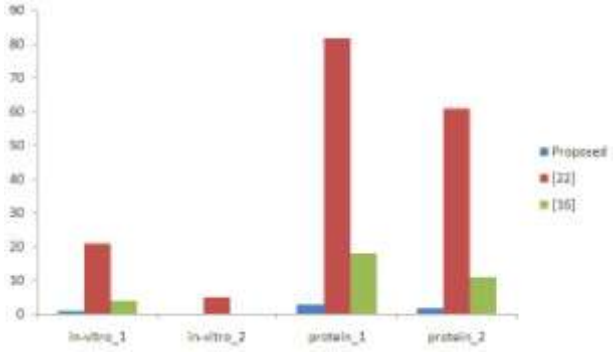

Figure 15. Graphical comparison on Table V

Test	#E		
	Proposed	[22]	[16]
in-vitro_1	252	351	621
in-vitro_2	197	281	423
protein_1	1933	2213	3215
protein_2	3977	1362	1574

Table VI. Comparison of Proposed Method with [16] and [22] for #E

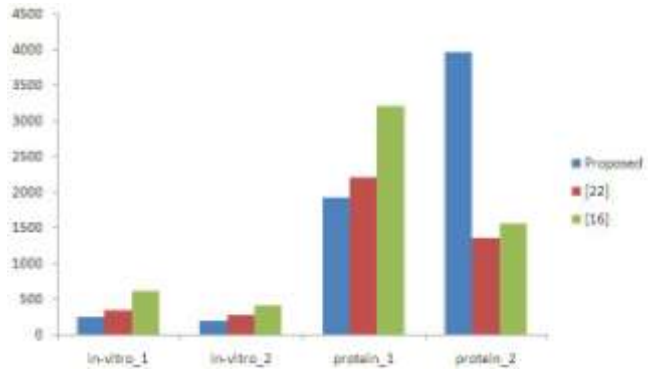

Figure 16. Graphical comparison on Table VI

Test	LaTime		
	Proposed	**[22]**	**[16]**
in-vitro_1	174	193	268
in-vitro_2	168	191	224
protein_1	1239	1394	1508
protein_2	1048	1108	1287

Table VII. Comparison of Proposed Method with [16] and [22] for *LaTime*

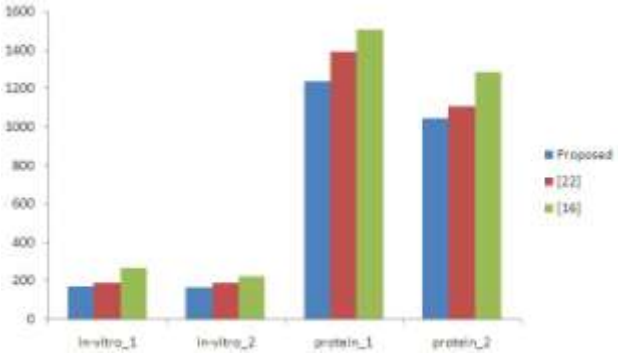

Figure 17. Graphical comparison on Table VII

The droplet clustering based cross-contamination avoidance method is further applied on real life protein assays. We have synchronized washing steps with functional droplet routing steps for harder sub-problems of protein synthesis. In table VIII the result is given along with the results of [20] for comparison where N_{CS} represents number of contaminating sites and N_{WOP} denotes number of wash operations required using intersection oriented wash droplet scheduling. The comparison establishes that the number of contamination in the proposed method is very less which subsequently results in less requirement for wash operations.

Sub-problem	**Proposed Method (Sec. V)**		**[20]**	
	N_{CS}	**N_{WOP}**	**N_{CS}**	**N_{WOP}**
78	0	0	1	1
79	0	1	0	3
80	1	0	3	5
81	0	2	5	4
82	2	0	4	3
83	0	1	3	1
84	1	0	1	4
85	0	0	4	0
86	0	0	0	3
87	0	0	3	0

Table VIII. Comparison of Proposed Method (Section V) with [20] on protein synthesis

VII. CONCLUSION

In this paper, we have discussed a staircase partitioning based droplet routing algorithm first. Then a droplet clustering based method for cross-contamination avoidance between large numbers of droplets clustered in sub-problems for DMFB has been proposed. The staircase partitioning based approach has certain limitations to manage cross-contaminations between two consecutive sub-problems. But the method is quite effective in managing intra-sub-problem cross-contaminations. Accordingly, a new droplet clustering method has been proposed here. This method effectively separates the serviceable droplets of a sub-problem in to some clustered areas. Thereafter it tries to assign the droplets of subsequent bioassay in to some clusters so that the whole chip area can be efficiently used during routing with minimum intersection and optimum routing completion time. In the experimental simulations, we have seen that both of our proposed methods are working better than some of the existing techniques. We have also observed that newly proposed technique in this paper is even better than the staircase partitioning approach. Droplet clustering based method has been extensively simulated for cross-contamination minimization between any two consecutive assay operations and also for harder sub-problems on protein synthesis. Experimentally, we have recorded better results in all such simulations in compare to some existing state of the art methods.

A significant observation on experimental simulation is that the proposed droplet clustering method is routing the droplets with minimum possible intersections without compromising the electrode usage and latest arrival time.

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