A Multi-path Droplet Routing Protocol for Digital Microfluidic Biochip

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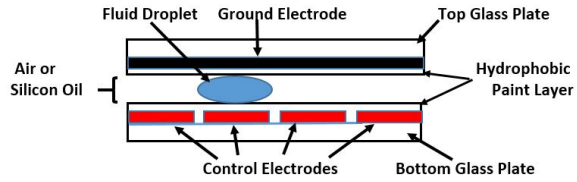
Digital microfluidic biochip provides an alternative platform to synthesize the biochemical protocols. Droplet routing in biochemical synthesis involves moving multiple droplets across the biochip simultaneously. It involves challenges like moving droplets without violating the fluid constraints. This article proposed a multi-path droplet routing protocol. Multiple routes are explored and validated using special packets. These routes are then classified based on user-defined heuristics. A single route is sequentially selected for each droplet. The routes are then compacted to generate a parallel moving sequence. The simulation result shows 2.087% and 4.952% improvement in the latest arrival time for free and virtual topology, respectively.

Povzetek: Članek predstavlja protokol za usmerjanje kapljic v digitalnih mikrofluidnih biočipih, ki omogoča izboljšave prihoda kapljic.

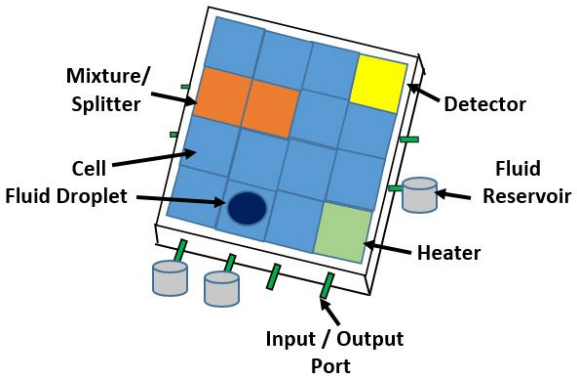
1 Introduction

Traditional medical diagnostics laboratories suffer from sample contamination as the samples need to be brought to the lab. During transportation, the samples may get contaminated due to mishandling. It leads to delays in analysis reports, resulting in limited usability in critical care applications [1, 2]. Other issues include chemical wastage, maintenance, immovability, etc. Digital microfluidic Biochips (DMFB) or Biochips can revolutionize the point-of-care device industry. DMFB is a handheld device that can transported to homes or chambers of elderly patients for collecting fluid samples such as blood, sweat, urine, etc. These samples are analyzed, and the result can be availed on the spot [3, 4]. DMFB's key features are portability, precision analysis, re-usability, space multiplexing, and dynamic configuration. The configuration of a biochip is shown in Fig. 1. DMFB consists of two glass plates held over each other. Either silicon oil or air fills the space between them. The fluid droplets are transported in this gap. Both the glasses are painted with hydrophobic paints to prevent the sticking of fluid droplets. Fig. 1(a) depicts the top view of the microfluidic Biochip. The top glass contains a single large ground electrode. The bottom glass plate has multiple control electrodes placed in a grid manner. Each control electrode is called *cell* and used for residing fluid droplets on it [5–7]. DMFB works based on the electrowetting principle [8, 9]. The electrode beneath the adjacent cell is powered to stretch the droplet toward the adjoining cell. The electrode beneath the current cell is switched off, resulting in the droplet crawling to the adjacent cell. Multiple cells are grouped together to form a mixture/ splitter module. Specialized modules such as a heater and detector need to be fitted with special cells. Various input and output ports reside on the periphery of the chip. Fluid reservoirs to store samples or waste are attached to these ports. Fig. 1(b) presents the side view of the Biochip. Biochemical synthesis using DMFB is performed in three stages, i.e., scheduling, placement, and droplet routing [10, 11, 18]. The biochemical protocols are given as input as a directed acyclic graph. In the scheduling stage, all the operations in the DAG are assigned a start and end time. In the placement stage, various modules like heaters, detectors, mixture or splitters, etc., are allocated specific locations on the biochip [12–15]. Both scheduling and placement stages are intertwined to have minimal droplet movement. The final stage deals with droplet routing. In this stage, the bio-assay is executed physically by moving fluid droplets between various droplet modules [16, 18]. Droplet routing in DMFB problem belongs to NP-hard family [5]. Various routing algorithms are proposed for both online and offline DMFB systems. In this article, a new multi-path routing protocol is proposed. The route exploration is performed by using duplicate *route request packets*. The routes are then verified by using *hello packets*. Multiple routes are explored and classified based on user-defined heuristics. A single route for each droplet is selected. The routes of all the droplets are compacted to generate a parallel moving sequence. Experimental result shows significant improvement in the latest arrival time.

This article is organized as follows: section II discusses the droplet routing problem in the digital microfluidic biochips. Section III presents a summary of various droplet routing protocols proposed in the past. Section IV describes the proposed protocol in detail. Experimentation and performance analysis are presented in section V. Finally, conclu-



(a) Sideview



(b) Topview

Figure 1: Configuration of digital microfluidic biochip [20, 26]

sion and future work is given in section VI.

2 Droplet routing

In the droplet routing stage, the aim is to execute a specific biochemical assay physically. Fluid operations are implemented by moving the fluid droplets from their source to their destination cell. Various challenges in droplet routing include droplet generation and droplet movement by avoiding unwanted mixing. It is assumed that the biochip hardware handles fluid droplet generation, but this is out of the scope of this work. Fuild constraints are defined to avoid unwanted mixing; they tend to mix when two fluid droplets become adjacent. It may result in erroneous experimental results. A biochemical protocol is represented by a directed acyclic graph (DAG). Nodes represent biochemical operations such as mixing, splitting, heating, etc., and an edge represents the interdependency among various operations. Three stages of biochemical synthesis using a digital microfluidic biochip are shown in Fig 2. In the scheduling phase, operations such as I_1, I_2, M_1, and O_1 are assigned a period for execution. In the placement phase, a module containing four cells, i.e., M_1, M_2, M_3, and M_4, are allocated locations on the biochip. The scheduling should be done so that the total number of modules available should always be greater than or equal to the total requirement by the operations [7, 16, 18, 25].

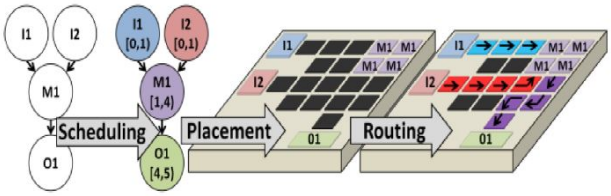


Figure 2: Stages of biochemical synthesis using DMFB [5]

2.1 Fluidic constraint

To avoid the unwanted mixing of two droplets due to adjacency, fluid constraints are defined. In other words, a space of at least one cell must be maintained between two droplets at any given time. To achieve this, a *bound box* is defined across the droplet. A bound box is a 3×3 window with the droplet residing on the center cell [17, 19].
Mathematically, the droplet routing problem can be defined as
Input: A m×n biochip with B blockages, a set of k droplets D. Each droplet D_i, have a source cell (X_i^s ,X_i^s) and a destination cell (X_i^d,Y_i^d) where i ≤k.
Output: A set of route R containing a route r_i for for every droplet D_i.
Constraint : The fluidic constraint has to be maintained at every instant of time.

3 Literature survey

Bohringer [21] introduced droplet routing in DMFB and proposed a solution based on the A^* algorithm. Su et al. [1] demonstrated the unwanted mixing due to the adjacency of two droplets. Many authors proposed droplet routing protocols for digital microfluidic biochips. It can be classified into four groups, i.e., VLSI-based, network flow-based, graph-based, and swarm intelligence-based. VLSI-based works have been proposed by Su et al. [1], Yu et al. [3], and Xu et al. [4]. The divide and conquer approach is used by Su et al. [1] to generate collision-free paths. However, it can run small assays (PCR, In-vitro). Frequent reconfiguration of modules and route exploration lead to failure to execute large assays (Protein-split). Yuh et al. [3] proposed solutions based on integer linear programming. It has limited formulations for the subproblems. It reduced the number of successful execution of a few assays. Works based on clique partition are proposed by Xu et al. [4]. It uses vertex and edge-disjoint routes. Due to the unavailability of free cells, it fails to run dense assays. Network flow model-based solutions are presented by Huang et al. [12] and Grissom et al. [5]. Huang et al. [12] use vector analysis to generate routing tracks based on the directions of flow of the droplets. The droplets moving in the same direction are sent on the same track. Grissom et al. [5] restrict the droplet flow rate to avoid congestion problems. Both the works [12], [5] suffer from the bottleneck of placement of modules, droplet

injection rate, or storage issues, which leads to complacency in converting routing problems in flow models. Roy et al. [6] and Swain et al. [25] present graph-based solutions. Concurrent routing of multiple droplets is used by Roy et al. [6] to generate a parallel moving sequence. The use of a large number of stalls leads to higher execution times for larger assays. Swain et al. [25] convert the routing problem into a rectangle overlapping problem. It was designed for free topology, so it fails to run most of the assays in virtual topology. A bidirectional routing protocol is proposed in their subsequent work, [26]. Pan et al. [7] and Juarez et al. [11] provide swarm intelligence-based solutions. Both the works [7], [11] are able to run all benchmarks, but their execution time is very high. So, these protocols apply to offline systems. All these works are designed primarily for a specific application-oriented task. Routers like Su et al., Roy et al., Grissom et al., Swain et al., and Huang et al. designed for online systems that need faster execution time, not optimal cell usage. Pan router and Juarez are designed for offline systems. The main aim of these types of protocols is to have optimal cell usage, not on the execution time. Hence, there is a need to develop a routing method that works well for both types of systems. In this work, the aim is to design an algorithm that will have optimal value for both online and offline systems.

4 Proposed routing method

The proposed method is based on the popular MMSPEED routing protocol for wireless sensor networks [23] and CMMSPEED [24]. The following assumptions are made in order to apply MMSPEED to droplet routing in DMFB.

- In MMSPEED, The location of every node in WSN may change. For DMFB, every cell is considered as a node and fixed. In other words, all nodes are static.
- In MMSPEED, the number of RR messages forwarded by the intermediate can be more than 4. Since DMFB is a rectangular grid, the maximum forwarded node limit is 3.
- The heuristics parameter, a number of intermediate nodes, is used instead of hub count.
- Two data packets can be adjacent in MMSPEED, but in DMFB, we need to maintain a gap of at least one cell between two droplets.

4.1 Algorithm

The proposed method has three phases, i.e., route exploration, route selection, and route compaction. In the route exploration phase, a route request (RR) packet is sent to every neighboring cell, excluding the corner cells. It is done to implement the restriction of movement to diagonal cells in DMFB. The address of every visited cell is added to the header field of the RR message. If the current cell visited is not the target cell, one copy of the RR message is again broadcast to all its adjacent cells, i.e., top, left, right, and bottom cells. If a node receives an RR packet with the same source, then it checks the RR's route field. If the route contains its address, then it assumes this RR packet has already been traversed by this node/cell. Hence, it discards the RR. This broadcasting of RR messages continues until one packet reaches the destination cell. After receiving the next four RR messages, all with the same source are discarded from the rectangular grid. The number of RR messages to store in the destination cell is user-defined and depends on the grid's reliability. For this work, the number of RR messages with the same point of origin is fixed to be 5. When an RR message reaches its destination cell/node, its header field is extracted and sent to the route explored. This extracted route is then validated by sending a *hello message* in the route's reverse direction, i.e., from the destination cell to the source cell. The algorithm for the exploration phase is given in Algorithm 1. In the next phase, i.e., route selection, all five routes are evaluated by heuristics like the number of intermediate cells or length and traversal time. Each route is then allocated priority. The shortest length route will have higher priority, and the longest route will have the lowest priority. Similarly, congestion along a route could be used to assign priority to a route. The routes are then sorted in descending order of priority. The final phase is route compaction. It is initiated after all droplet routes have been explored, validated, and classified. The goal of this phase is to generate a parallel moving sequence. All the droplets are moved along their best route, one cell per cycle. If any droplet violates fluid constraints in the next time cycle, then a halt cycle is added. It is done to prevent the deadlock. It will work in intersecting routes but not in parallel routes where droplets are moving towards each other. This scenario is handled by detouring, i.e., using alternative routes from that point. If any such sub-path exists, then it is used. Else. Compaction is again performed using the second-best path.

5 Experimentation and result analysis

The proposed method's performance is evaluated against eight droplet routing protocols. The experimentation involves thirteen test cases of standard biochemical benchmarks [1]. The simulation is performed using a UCR microfluidic static simulator [27] running on a 64-bit Windows 10 desktop PC with 16 GB of RAM and an Intel Core i5™ CPU operating at 2.8GHz. The UCR microfluidic static simulator was an open-source developed at the University of California, Riverside. The front end, i.e., graphical interfaces, is created using JAVA, and its back-end operations are implemented using C++. The scheduling phase uses path scheduler [28]. Krammer placer [28] is utilized to assign positions of various modules on the chip. The biochemical assay benchmarks are classified into three groups,

Algorithm 1: : Algorithm for path exploration

Input : A a×b biochip, with c blockages, a set of n droplets D, where each droplet D_i have source cell (X_i^s ,Y_i^s) and a destination cell (X_i^d ,Y_i^d) for 0 < i ≤n

Output: A set of routes R containing set of n route list, where. R_i is the route list of droplet D_i for 0 < i ≤ n

```
for Every D_i, i = 0 to n do
    Send a Route Request Packet RR_i to all the adjacent cells of X_i^s ,Y_i^s
    while RR_i has not reach the cell (X_i^d ,Y_i^d) do
        RR_i is at cell (X_i^c ,Y_i^c)
        if Header of RR_i don't contains (X_i^c ,Y_i^c) then
            Add (X_i^c ,Y_i^c) to its header
            Forward RR_i to adjacent cells of (X_i^c ,Y_i^c)
        else
            Discard RR_i
        end if
    end while
    When a RR_i reaches (X_i^d ,Y_i^d)
    Extract route_k from header of RR_i,0 < k ≤ p, where p the number of routes required
    Send an HELLO_i^k packet from (X_i^d ,Y_i^d) to (X_i^s ,Y_i^s) along route_k
    when HELLO_i^k reach (X_i^s ,Y_i^s), save the path route_k into validated route list.
end for
```

i.e., PCR, In-vitro diagnostics, and a protein assay. The dimension of the biochip is fixed at 38 × 42. The droplet generation rate or actuation frequency is set to 100 Hz [5]. All the protocols are executed in two topologies, i.e., free and virtual topology. The free topology simulates the ideal scenario with no blockages. The virtual topology emulates realistic scenarios with blockages [29]. The performance of the proposed method is compared using the latest arrival time (LAT) parameter with other routing protocols. The LAT refers to the period at which the last droplet of the executed assay reaches its destination.

5.0.1 Latest arrival time

The performance study of the proposed method is performed in two phases. In the first phase, the biochip performance is considered in an ideal scenario. So, the biochip with free topology was selected for simulation. The efficacy of the proposed method is compared using the parameter latest arrival time or total execution time. The LAT is measured in milliseconds. Table I presents the simulation result of the test runs in free topology.

All the protocols can run every benchmark successfully. Su router [1] has the best LAT value for the PCR benchmark only. Cho router [2] performs better than Su router apart from PCR. It has the best LAT for In-vitro 3 assay and near minimal value for In-vitro 3 and Protein split 1. Roy router [6] has the lowest LAT value for the In-vitro 1 benchmark and near-optimal for the Protein split 3 benchmark. Grissom router [5] has optimal LAT value for Protein split 1 and approximately optimal LAT value for In-vitro 2 and 3. Swain router [25] has suboptimal LAT values for PCR, In-vitro 3 benchmarks. Huang router [16] has the lowest LAT value for the Protein split 2 benchmark and approximately the lowest LAT for PCR, In vitro 2 and 3. Both the swarm intelligence-based protocols, i.e., Pan router [7] and Juarez router [11], are also able to run all the benchmarks. Both of the protocols have higher LAT values compared to other protocols. The proposed protocol is also able to run all the benchmarks. It has the optimal LAT value for In-vitro 3, 4, and 5; Protein split 3,4,5,6 and 7.

The next simulation's goal is to study the performance behavior of the proposed method in a real-world scenario; for this, a biochip with virtual topology architecture with 10% blockages is considered. The distribution of blockages is done randomly in such a way that the rectilinear graph remains connected. The graph connectivity test is done first, if successful, execution of a benchmark is initiated. The performance of a benchmark depends on the distribution of blockages. To minimize this effect, ten distributions of the blockage configuration is considered for the study. The average of all the 10 test runs of the simulation result is presented in Table 2.

Su router [1] can run most of the assays except Protein split 5, 6, and 7. Cho router [2] successfully executed all the bioassays. It has the lowest LAT for the PCR benchmark. It performs better than the Su router for all benchmarks apart from the Protein split 1 benchmark. Roy router [6], Grissom router [5], and Huang router [16] able to run all the benchmarks. Roy router [6] has the lowest LAT for the In-vitro 4 benchmark. Grissom router [5] has minimum LAT for Protein split 1 benchmark. Huang router [16] has the best LAT for In-vitro 1 benchmark. Swain router [25] executed most of the benchmarks except Protein split 4, 5, 6, and 7. The swarm intelligence-based protocols also successfully executed all the benchmarks. In most test cases, these protocols have higher LAT than other protocols. Pan router [7] and Juarez router [11] router have minimal LAT for In-vitro 2 and In-vitro 5 benchmarks, respectively. The proposed protocol has optimal LAT value for In-vitro 3, Protein split 2, 3, 4, 5, 6, and 7.

5.1 Analysis of proposed algorithm

In free topology, Lee's router [30] is used by Su router [1] and Roy router [6] for route exploration. Similarly, the Grissom router [5] uses an improved Lee router, i.e., Soukup's router [31] for the exploration of the route for droplets. Both these routers are fast and guarantee to find a route. As the assay size becomes large, the routes are either partially or fully shared by multiple routes. During the compaction phase, a large number of stalls or detours are

Benchmark	Su2006 [1]	Cho2008 [2]	Roy2010 [6]	Grissom2014 [5]	Swain2019 [25]	Huang2020 [16]	Pan2011 [7]	Juarez2018 [11]	Proposed
PCR	**371**	389	422	415	396	382	502	582	387
In-Vitro 1	527	503	**450**	512	540	552	617	725	462
In-Vitro 2	702	**522**	825	563	615	578	643	813	548
In-Vitro 3	924	735	1137	750	749	740	1055	1122	**724**
In-Vitro 4	1263	1143	1408	1264	1064	1265	1194	1207	**956**
In-Vitro 5	2243	1457	2647	1583	1533	1657	1487	1466	**1231**
Protein Split 1	526	443	764	**427**	511	493	521	634	442
Protein Split 2	1237	1215	1243	1462	1382	**1084**	1305	1376	1163
Protein Split 3	7701	6786	6015	6389	7631	6358	6093	7528	**6057**
Protein Split 4	15223	14280	14813	16756	15073	18811	16517	17091	**13537**
Protein Split 5	39327	34971	42088	37944	39689	36990	33093	34675	**30521**
Protein Split 6	83675	70168	69423	78022	82707	78323	65902	68496	**66813**
Protein Split 7	205362	183253	182511	101735	199413	183542	159393	162637	**143417**

Table 1: Latest arrival time in free topology

Benchmark	Su2006 [1]	Cho2008 [2]	Roy2010 [6]	Grissom2014 [5]	Swain2019 [25]	Huang2020 [16]	Pan2011 [7]	Juarez2018 [11]	Proposed
PCR	829	**579**	924	663	580	596	627	653	588
In-Vitro 1	815	736	840	926	802	**646**	665	683	662
In-Vitro 2	955	850	960	1153	1045	705	**694**	697	702
In-Vitro 3	1561	1409	1781	1716	2068	1116	1132	1178	**1088**
In-Vitro 4	2221	2146	**1846**	2173	2307	2349	2145	2236	1808
In-Vitro 5	6284	6193	5192	4427	6093	4827	4736	**4357**	4442
Protein Split 1	1068	1254	1115	**990**	1074	1024	1065	1125	991
Protein Split 2	2629	2063	2441	2739	2389	2565	2213	2121	**2013**
Protein Split 3	9342	7453	5890	6197	9326	5962	6175	5971	**5786**
Protein Split 4	20195	17985	16388	15960	Fail	14549	15756	14750	**13438**
Protein Split 5	Fail	47620	35367	34961	Fail	36534	34287	32904	**26231**
Protein Split 6	Fail	89265	793602	76573	Fail	77586	75001	74475	**59929**
Protein Split 7	Fail	346512	293779	274406	Fail	223645	235306	231693	**196000**

Table 2: Latest arrival time in virtual toplology

used to generate a parallel movement sequence. For larger assays, this issue becomes much more complex and often fails. This is the reason for the failure to run all dense assays. Deadlock-free routers like Grissom routers are able to run all benchmarks due to the controlled injection rate of droplets. Cho router performance depends on the creation of a concession zone to temporarily store one of the droplets in deadlock. As the size of the assay increases, finding free cells to create concessions becomes very difficult. This is the reason behind the failure to execute large assays. The swarm intelligent-based routers need initial population generation and selection of the best candidates to populate the next generation of potential candidates. This process requires more processing time. Hence, these two protocols have a higher latest arrival time than other protocols. The method is used to store the small segment of routes during the route exploration phase. During the compaction phase, when the number of stalls exceeds 4, detouring is used to handle deadlock. The value four is taken from the Grissom router. The use of small sub-routes to bypass the congestion zone improves the performance. All the other protocols initiate the route exploration phase again to find alternate routes for detouring. It acts as an overhead and contributes to a higher execution period and failure of execution. The proposed protocol performs route exploration only once and stores the sub-routes. Due to the storage of sub-routes, the memory requirement is higher than that of all other protocols.

Equation 1 is used to measure the performance of the proposed method in both topologies.

$$Improvement = \frac{LAT_{others} - LAT_{Proposed}}{LAT_{others}} \times 100 \quad (1)$$

$LAT_{Proposed}$ represents the LAT value of the proposed method, while LAT_{others} refers the best performing protocol among other protocols. It has been observed that an overall average improvement of 2.087% in latest arrival time for free topology. It is also noted an improvement of 16.03% for In-vitro 5 protocol. Similarly, for virtual topology, an overall average improvement of 4.952% in latest arrival time. It also records an improvement of 19.531% for Protein split 6 benchmark. It is noticed that the proposed protocol performs better in virtual topology than free topology. For small assay traditional methods performs better than the proposed method. For larger assays, the proposed method performance improves as the assay size increases and reach a threshold point (for protein split 5) and then decreases gradually.

6 Conclusion

Digital microfluidic biochip is a recent trending research area. Biochemical synthesis is performed using digital microfluidic biochip. Droplet routing is one of the essenial phase in the synthesis. The goal is to transport various droplets from its source cell to its destination cell maintaining fluidic constraint at every time interval. In this article, a multi-path droplet routing protocol is proposed. In the exploration phase, duplicate *route request packets* are used to discover the routes. Then *hello packets* are used for validating the discovered route. In the classification phase, routes are grouped by the route length heuristic and sorted in ascending order. Then compaction is performed to get the parallel moving sequence. Simulation result shows 2.087% and 4.952% improvement in latest arrival time. In future scope of this article is to study the effect of distribution of blockages on number of shared cells and latest arrival time.

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