Synchronization of Washing Operations with Droplet Routing for Cross-Contamination Avoidance in Digital Microfluidic Biochips*

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ABSTRACT

Digital microfluidic biochips are being utilized in many areas of biochemistry and biomedical sciences. Since cross-contamination between droplets of different biomolecules can lead to erroneous outcomes for bioassays, it is essential to avoid cross-contamination during droplet routing. We propose a wash-operation synchronization method to manipulate wash droplets to clean the residue that is left behind by sample and reagent droplets. We also synchronize wash-droplet routing with sample/reagent droplet-routing steps by controlling the arrival order of droplets at cross-contamination sites. The proposed method minimizes droplet-routing time without cross-contamination, and it is especially effective for tight chip-area constraints. A real-life application is used for evaluation.

Categories and Subject Descriptors

B.2.2 B.2.m [**Hardware**]: Performance analysis and design aids.

General Terms

Algorithms, Performance, Design.

Keywords

Droplet-based microfluidics, electrowetting, lab-on-chip.

1. INTRODUCTION

Droplet-based "digital" microfluidics is an emerging technology that aims to miniaturize and integrate fluid handling on a chip [1, 4]. Several complex biomedical procedures have recently been demonstrated on the digital microfluidics platform, e.g., gene sequencing through synthesis [1], protein crystallization for drug discovery [15], and cell sorting [7]. These advances in technology and applications serve as a powerful driver for research on computer-aided design (CAD) tools for biochip design. A number of CAD methods have been developed recently for the design and use of microfluidic biochips [2, 5, 8, 10, 14, 18, 19].

In many biomedical assays, liquids that contain large molecules such as proteins are transported on a microfluidic platform [1]. However, proteins cannot be transported easily since they tend to adsorb irreversibly to hydrophobic surfaces and contaminate them [1, 9]. When multiple droplet routes intersect with each other, a droplet that arrives at a later clock cycle at the intersection can be contaminated by the residue left behind by another droplet that passed through at an earlier clock cycle. Thereby, cross-contamination occurs at this intersection, and it is called a "cross-contamination site". Cross-contamination must be avoided during droplet routing since it leads to erroneous assay outcomes.

Wash operations are used to avoid cross-contamination between different droplet routes. Wash droplets traverse the cross-contamination sites and clean residue between successive functional droplet routing. In [6], a washing step is necessary after each polypyrrole synthesis step to avoid cross-contamination in the DNA biochip. Wash droplets are also utilized in the clinical application of diagnosis for Huntington's disease [12].

In this paper, we integrate washing steps into droplet-routing steps for the target bioassay to alleviate the cross-contamination problem. We refer to the routing of sample and reagent droplets as *functional droplet routing*. Wash droplets are manipulated to traverse all the cross-contamination sites and clean the residue left behind by functional droplets. The routing of wash droplets is synchronized with functional droplet-routing. This approach leads to reduced droplet-transportation time. Compared to prior work, the advantage of the proposed method for reducing droplet-transportation time is more striking for smaller chip area. The cost of disposable biochips is determined by the chip footprint and packaging, therefore small chip area is desirable.

The remainder of the paper is organized as follows. Section 2 provides an overview of digital microfluidics and related prior work on droplet routing. Section 3 describes the synchronization of wash-droplet routing with functional-droplet routing. In Section 4, two baseline methods that incorporate washing steps in the functional droplet-routing steps are described. In Section 5, a real-life bioassay is used to evaluate the proposed method. Finally, conclusions are drawn in Section 6.

2. DIGITAL MICROFLUIDICS AND RELATED PRIOR WORK

In digital microfluidics, droplets of nanoliter volumes are manipulated on a two-dimensional electrode array [1]. A unit cell in the array includes a pair of electrodes that acts as two parallel plates. The bottom plate contains a patterned array of individually controlled electrodes, and the top plate is coated with a continuous ground electrode. A droplet rests on a hydrophobic surface over an electrode. Fig. 1 shows a recent prototype chip for protein crystallization and multiplexed immunoassays.

Droplets are moved by applying a control voltage to a unit cell adjacent to the droplet and, at the same time, deactivating

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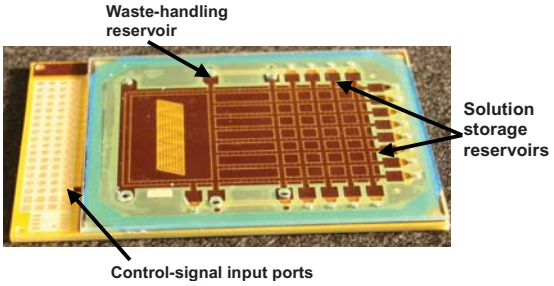

Figure 1: A fabricated digital microfluidic array [3].

the one under the droplet. This electronic method of wettability control creates interfacial tension gradients that move the droplets to the charged electrode. Fluid-handling operations such as droplet merging, splitting, mixing, and dispensing can be executed in a similar manner. Droplet routes and operation schedules are programmed into a microcontroller that drives the electrodes.

In order to solve the droplet-routing problem for digital microfluidics, a number of techniques have been proposed [5,10,14, 16,18,19]. However, the droplet routes produced by these methods may overlap with each other, giving rise to the problem of cross-contamination. In [17], a droplet-routing method is described to minimize the likelihood of cross-contamination in the optimization of droplet flow paths. This approach targets disjoint droplet routes to avoid overlap between different droplet routes. However, this method suffers from several drawbacks. First, more electrodes are used for disjoint droplet routes than for non-disjoint droplet routes, thereby the length of droplet routes is increased. Since some functional droplets have to detour along the disjoint routes, the droplet-transportation time is also increased. Second, the primary goal of this method is to find a set of vertex-disjoint routes that completely avoids the overlap between different droplet routes. If the primary goal fails, this method tries to find a set of edge-disjoint routes as a design compromise. Since the edge-disjoint routes have no common pair of adjacent cells but may still share a single cell, the avoidance of all cross-contamination sites cannot be guaranteed.

If cross-contamination sites cannot be completely avoided by disjoint routes, wash operations are used to avoid cross-contamination [6, 12, 16, 17]. Consider an intersection of two functional droplet routes. When a droplet passes through the intersection, a wash droplet is dispensed from the wash reservoir and transported via this intersection to the waste reservoir. The wash droplet will clean the residue at this intersection left behind by the earlier droplet. After that, the other droplet is transported from the source node via this cleaned intersection to the sink node. In this manner, cross-contamination between the functional droplets can be avoided. For liquids that contain large molecules such as proteins, one wash droplet is sometimes not sufficient to clean a cross-contamination site. In this situation, multiple wash droplets have to be used for each cross-contamination site.

In [17], washing steps are simply inserted between successive functional droplet-routing steps, e.g., by using trailer wash droplets for sample droplets. In this situation, the completion time for droplet routing is the sum of the transportation time for both functional droplets and wash droplets, which is much higher than the droplet-routing time without washing steps. For smaller chip area, since there are fewer free cells available for droplet routing, it is more difficult to obtain disjoint droplet routes; therefore, the number of cross-contamination sites increases. In this case, more washing steps have to be inserted,

Table 1: Adjustment of droplet-arrival order.

Droplet arrival order			
D_1	D_2	W	Adjustment
1	2	3	D_2 is made to arrive after W traverses the site
1	3	2	Maintain this order
3	1	2	Maintain this order
3	2	1	W is made to arrive after D_2 traverses the site
2	1	3	D_1 is made to arrive after W traverses the site
2	3	1	W is made to arrive after D_1 traverses the site

which further increases the droplet-routing time. Therefore, there is a need to synchronize wash-droplet routing with functional droplet-routing steps, in order to reduce the time needed for droplet routing, and at the same time completely avoid cross-contamination. A contamination-aware droplet routing algorithm is proposed in [16], where a minimum cost circulation algorithm and look-ahead prediction are used to optimize wash-droplet routing. This technique iteratively compacts the routing paths of wash droplets with previously compacted routing paths of functional droplets without optimizing the order in which multiple cross-contamination sites are considered. Therefore, the droplet-transportation time with washing steps is not minimized.

3. SYNCHRONIZATION OF WASH-DROPLET ROUTING WITH FUNCTIONAL DROPLET ROUTING

Since a digital microfluidic array can be reconfigured dynamically, the droplet-routing problem is decomposed into a series of sub-problems [14]. In each sub-problem, the fluidic ports on the boundary of microfluidic modules are referred to as *pins*. The droplet routes between pins of different modules or on-chip reservoirs are referred to as *nets*. The modules that are active at the time when droplets are transported are considered as *obstacles*. We obtain a complete droplet-routing solution by solving these sub-problems sequentially.

Within one sub-problem, there exist multiple sites where different droplet routes intersect with each other. We first address the problem of synchronizing wash-droplet routing with functional-droplet routing for one cross-contamination site, and extend the solution to synchronization for multiple cross-contamination sites.

3.1 One Cross-Contamination Site

The proposed synchronization of wash-droplet routing with functional-droplet routing for one cross-contamination site includes two steps. In the first step, we generate the route for the wash droplet (or multiple wash droplets) to traverse the cross-contamination site. This droplet route consists of two sub-routes. The first sub-route connects the dispensing reservoir for wash droplets (source) to the cross-contamination site (sink), and the second sub-route connects the cross-contamination site (source) to a waste reservoir (sink). A droplet-routing method based on [14] is utilized to generate two sub-routes.

For one cross-contamination site, the routing of the wash droplet (or multiple wash droplets) is synchronized with the routing of two functional droplets. These droplets start from the corresponding sources at the same time and are transported along the computed droplet routes via the cross-contamination site towards the sinks. The arrival time of the wash droplet at the cross-contamination site depends on the length of the

first sub-route. Note that the arrival time of a droplet from the source to the sink is calculated based on the length of the corresponding route. If a droplet is transported across one electrode per clock cycle, the arrival time of the droplet is equal to the number of electrodes in the route. The wash droplet (or multiple wash droplets) will reach the site at an appropriate time, between the arrival times of the two functional droplets.

Therefore, in the second step, we adjust the arrival order of the wash and functional droplets, to ensure that the wash (or multiple wash droplets) arrives at the cross-contamination site later than one functional droplet, but earlier than the other. Without loss of generality, here we consider one wash droplet for a cross-contamination site. For a cross-contamination site S where the route of droplet D_1 intersects with the route of droplet D_2, we first estimate the arrival order of D_1, D_2, and the wash droplet W based on the lengths of droplet routes connecting their sources to the cross-contamination site, respectively. Next we adjust the arrival order of these three droplets according to Table 1. For example, if D_1, D_2 and W arrive at the cross-contamination site serially, we delay the arrival time of D_2 such that W traverses the site before D_2 arrives. In this way, the residue left behind by D_1 at an earlier clock cycle will be cleaned by the wash droplet W before D_2 arrives. Note that the droplet whose arrival time should be postponed is temporarily stored (held) in an on-chip storage unit. After the adjustment for one cross-contamination site is carried out, we record the updated time when droplets D_1, D_2 and W traverse this site. Note that if a droplet has to be stored in an on-chip storage unit, the total transportation time for this droplet is the sum of the droplet-routing time and the duration for the on-chip storage. Therefore, the duration for the on-chip storage of this droplet should be minimized.

Fig. 2 shows the synchronization of wash-droplet routing with functional-droplet routing for one cross-contamination site. As shown in Fig. 2(a), the droplet routes of two functional droplets D_1 and D_2 intersect at the cross-contamination site S. A wash droplet W is dispensed from the wash reservoir to clean the residue at S. The wash-droplet route consists of two sub-routes. The first sub-route connects the wash reservoir to S, and the second sub-route connects S to the waste reservoir. We estimate the arrival order of D_1, D_2, and W at the cross-contamination site S assuming that all the droplets start moving at the same time. From Fig. 2(a), based on the lengths of droplet routes connecting their sources to the cross-contamination site, we calculate that D_1 arrives at S at clock cycle 5, D_2 arrives at S at clock cycle 3, and W arrives at S at clock cycle 9. According to Table 1, we should delay the arrival time of D_1 such that W traverses the site before D_1 arrives. As shown in Fig. 2(b), at clock cycle 9, D_2 has arrived at the sink node, and W arrives at the cross-contamination site S to clean the residue left behind by D_2. D_1 is stored in the on-chip storage unit, instead of being transported along its droplet route. There is a one-electrode spacing between D_1 and W in order to avoid undesirable mixing. In Fig. 2(c), at clock cycle 13, W has left the cross-contamination site S and is transported to the waste reservoir, and D_1 arrives at S that has been cleaned. In Fig. 2(d), at clock cycle 17, three droplets D_1, D_2 and W have arrived at the corresponding destinations.

Therefore, the maximum droplet-transportation time is 17 clock cycles. We record the transportation-timing information when functional droplets D_1 and D_2 traverse the cross-contamination site S, i.e., D_1 traverses at clock cycle 13 and D_2 traverses at clock cycle 3. If D_1 or D_2 also traverses other cross-contamination sites in the same sub-problem, the transportation-timing information is utilized when we adjust the arrival order of wash droplets and functional droplets at these cross-contamination sites. Next we describe the synchronization of wash droplets and functional droplets for multiple cross-contamination sites in a sub-problem.

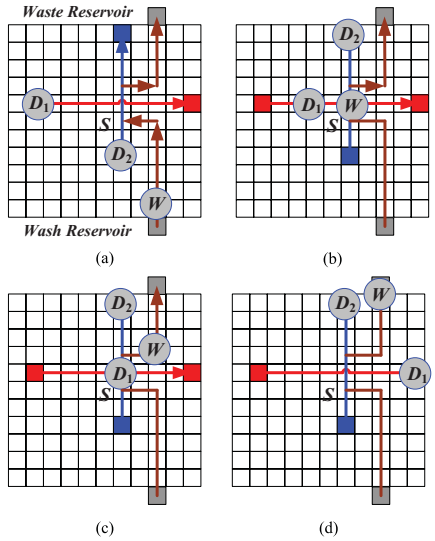

Figure 2: Synchronization for one cross-contamination site: (a) wash-droplet routes and functional-droplet routes; (b) snapshot at clock cycle 9; (c) snapshot at clock cycle 13; (d) snapshot at clock cycle 17.

3.2 Multiple Cross-Contamination Sites

Within one droplet-routing sub-problem, usually there are multiple cross-contamination sites where the routes for a pair of functional droplets intersect. Each cross-contamination site needs a wash droplet (or even multiple wash droplets) to clean the residue on it. Here we assume that one wash droplet is enough to clean one cross-contamination site. We propose to synchronize wash-droplet routing with functional-droplet routing for each cross-contamination site. If these cross-contamination sites are independent, i.e., a droplet route does not traverse multiple sites, we can simply utilize the synchronization method for one cross-contamination site (Section 3.1).

Next we consider the case that a droplet route intersects with multiple routes at different cross-contamination sites. For example, as shown in Fig. 3(a), the route of droplet D_1 intersects with the routes of droplet D_2 and D_3 at sites $S_{1,2}$ and $S_{1,3}$, respectively. Droplet D_1 is transported along its route to first traverse $S_{1,2}$ and then $S_{1,3}$. Two wash droplets, $W_{1,2}$ and $W_{1,3}$, are dispensed from the wash reservoir to clean the two cross-contamination sites $S_{1,2}$ and $S_{1,3}$, respectively.

To synchronize wash-droplet routing with functional droplet routing, first we generate the droplet routes for $W_{1,2}$ and $W_{1,3}$ to traverse $S_{1,2}$ and $S_{1,3}$, respectively, as shown in Fig. 3(b). As mentioned in Section 3.1, each wash-droplet route consists of two sub-routes. Next we adjust the arrival order of the wash droplet and two functional droplets for each cross-contamination site. For site $S_{1,2}$, we adjust the arrival order of $W_{1,2}$, D_1, and D_2, based on their estimated arrival order acquired from the lengths of droplet routes connecting their sources to site $S_{1,2}$. Similarly, for site $S_{1,3}$, we adjust the arrival order of $W_{1,3}$, D_1 and D_3.

Assume that we first adjust the arrival order for site $S_{1,3}$ and then carry out the adjustment for site $S_{1,2}$. Therefore, we adjust for site $S_{1,2}$ after the adjustment for site $S_{1,3}$. Since D_2, D_1 and $W_{1,2}$ arrive at $S_{1,2}$ serially based on the estimate of the droplet-route lengths from their sources to $S_{1,2}$, D_1 has

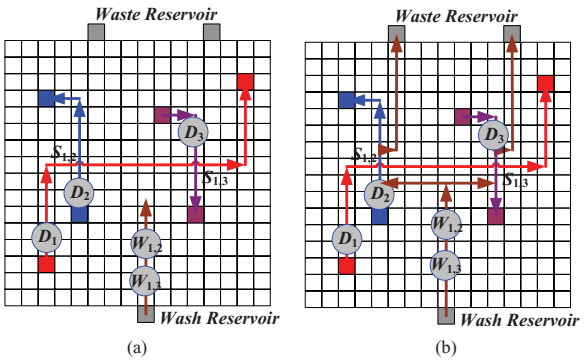

Figure 3: Synchronization for two cross-contamination sites: (a) functional-droplet routes and cross-contamination sites $S_{1,2}$ and $S_{1,3}$; (b) synchronization of wash-droplet routing with functional-droplet routing.

to be temporarily stored for several clock cycles. This has to be done to ensure that $W_{1,2}$ first passes through $S_{1,2}$; i.e., the time when D_1 passes through site $S_{1,2}$ is delayed. However, since the transportation timing of D_1 is modified at $S_{1,2}$, the arrival order of $W_{1,3}$, D_1 and D_3 at site $S_{1,3}$, which has been adjusted based on their initial estimate of the droplet-route lengths from their sources to site $S_{1,3}$, is not valid any more. Therefore, we have to adjust the arrival order for site $S_{1,3}$ again based on the updated transportation timing of D_1, which is obtained from the adjustment for $S_{1,2}$. A total of three adjustments have to be made, one for $S_{1,2}$ and two for $S_{1,3}$. If there are many cross-contamination sites within one sub-problem, the adjustment for one site may have to be repeated several times if we adjust all the sites without any carefully determined order.

However, if we first adjust the arrival order for site $S_{1,2}$, we can utilize the updated transportation timing of D_1 when we adjust for site $S_{1,3}$. A total of only two adjustments, one for $S_{1,2}$ and the other for $S_{1,3}$, will now be necessary.

Therefore, when synchronizing wash-droplet routing with functional-droplet routing for multiple cross-contamination sites, we have to adjust the arrival order for these sites in a predetermined order. This site-adjustment order is determined as follows: for a functional droplet that traverses multiple cross-contamination sites serially, we adjust the arrival order for these sites following the same order as this functional droplet traverses. Therefore, for each functional droplet in a sub-problem, we first list the cross-contamination sites that it traverses along the route from the source to the sink serially. Next we combine these lists for different functional droplets together into a site-adjustment order such that the order of these cross-contamination sites maintains the same as in their original lists. For example, in a sub-problem, the routes of four functional droplets, D_1 to D_4, intersect at four cross-contamination sites. $S_{i,j}$ is the cross-contamination site of routes for D_i and D_j. D_1 traverses $S_{1,3}$, $S_{1,4}$ and $S_{1,2}$ serially; D_2 traverses $S_{2,3}$ and $S_{1,2}$ serially; D_3 traverses $S_{2,3}$ and $S_{1,3}$ serially. We combine these lists together into a site-adjustment order: $S_{2,3} \rightarrow S_{1,3} \rightarrow S_{1,4} \rightarrow S_{1,2}$, where the order of these cross-contamination sites is the same as in their original lists. We adjust the arrival order for four sites following this site-adjustment order. After the adjustment for one site, we record the updated transportation timing when each functional droplet traverses the site. In this manner, when adjusting the arrival order for the next site, we can utilize the newly updated transportation timing information from the previously adjusted sites within the site-adjustment order.

The procedure for synchronizing washing steps with functional droplet-routing steps in a sub-problem is shown in Fig. 4.

1) **Step 1.** Generate droplet routes for the transportation of functional droplets;
2) **Step 2.** Determine the site-adjustment order: the arrival order will be serially adjusted at cross-contamination sites following the order;
3) **Step 3.** For each cross-contamination site following the site-adjustment order:
   i) 3(a): Generate the route for the wash droplet to traverse the cross-contamination site;
   ii) 3(b): Adjust the arrival order of the wash droplet and two functional droplets at the cross-contamination site;
   ii) 3(c): Update the transportation-timing information when each functional droplet traverses the cross-contamination site;
4) **Step 4.** Modify the transportation timing for functional droplets that do not traverse any cross-contamination site.

Figure 4: Procedure for synchronizing washing steps with functional droplet-routing steps in a sub-problem.

For an $M \times N$ microfluidic array, the worst-case computational complexity of the routing algorithm used to generate the route for a functional or wash droplet is $O(MN)$ [14]. The worst-case time complexity of the synchronization of washing steps with functional droplet-routing steps for k cross-contamination sites in a sub-problem is $O(kMN)$.

4. BASELINE APPROACHES

In order to establish the effectiveness of the proposed method to synchronize wash-droplet routing with the functional-droplet routing, we consider two baseline methods.

4.1 Baseline Method 1: Wash-Operation Insertion

The first baseline method inserts wash operations between successive functional-droplet routing steps within one sub-problem to clean the residue at cross-contamination sites. Note that wash droplets and functional droplets are not transported concurrently in this baseline case.

For example, for the droplet routes in Fig. 3(b), at first, D_1 is transported along its route to the sink node. After that, two wash droplets, $W_{1,2}$ and $W_{1,3}$, are dispensed from the wash reservoir to clean two cross-contamination sites $S_{1,2}$ and $S_{1,3}$, respectively. After $W_{1,2}$ and $W_{1,3}$ have cleaned $S_{1,2}$ and $S_{1,3}$ and are transported to the waste reservoirs, D_2 and D_3 are transported along their corresponding routes via the cleaned sites to the sink nodes. In this case, one wash operation is inserted between two successive functional-droplet routing steps.

For baseline method 1, the maximum droplet-transportation time for all the nets is the sum of the droplet-transportation time for wash-operation steps and functional-droplet routing steps.

4.2 Baseline Method 2: Appending Wash Droplets

The second baseline method appends one wash droplet to each functional droplet. For example, in Fig. 3(a), instead of using two wash droplets $W_{1,2}$ and $W_{1,3}$ to clean two cross-contamination sites $S_{1,2}$ and $S_{1,3}$, we append one wash droplet to each of functional droplets D_1, D_2 and D_3. During functional-droplet routing, the wash droplets follow the corresponding functional droplets and clean the residue.

For baseline method 2, the maximum droplet-transportation time for all nets can be divided into three parts. The first part is the droplet-transportation time for transporting all wash droplets from wash-droplet dispensing reservoirs to the source nodes of their corresponding functional droplets. The second part is the droplet-transportation time for moving all wash droplets from the sources nodes to the sink nodes following their corre-

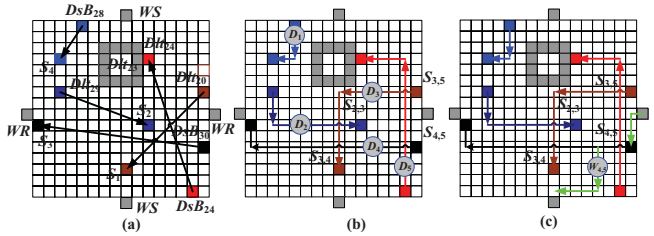

Figure 5: Synchronization of washing steps with functional droplet-routing steps in Sub-problem 82: (a) five 2-pin nets; (b) functional droplet routes; (c) the wash-droplet route for cross-contamination site $S_{4,5}$.

sponding functional droplets. The third part is the transportation time for moving all wash droplets to the waste reservoirs.

5. EXPERIMENTAL EVALUATION

In this section, we evaluate the proposed wash-operation synchronization method for a protein assay. We also compare it with two baseline methods and prior work [17].

5.1 Protein assay

We first evaluate the proposed method and two baseline methods for a real-life protein assay that has been carried out on a digital microfluidic lab-on-chip [13]. Based on the synthesis results using the method in [2], there are 198 fluidic operations (including storage units) for the protein assay. The routing problem is decomposed into 127 sub-problems. We synchronize washing with functional droplet-routing for each sub-problem.

For example, we synchronize washing steps with functional droplet-routing steps for Sub-problem 82. In Sub-problem 82, there are five 2-pin nets for the transportation of functional droplets, and Dlt_{23} is concurrently operating, as shown in Fig. 5(a). First, the proposed method generates droplet routes for the transportation of functional droplets using the routing method from [14]. As shown in Fig. 5(b), Droplet D_1 transports along the route from the buffer dispenser for DsB_{28} to an on-chip storage unit S_4; Droplet D_2 transports from the diluter for Dlt_{29} to an on-chip storage unit S_2; Droplet D_3 transports from the diluter for Dlt_{20} to an on-chip storage unit S_1; Droplet D_4 transports from the buffer dispenser for DsB_{30} to an on-chip storage unit S_3; Droplet D_5 transports from the buffer dispenser for DsB_{24} to the diluter for Dlt_{24}. The routes of four functional droplets, D_2 to D_5, intersect at four cross-contamination sites. $S_{i,j}$ is the cross-contamination site of routes for D_i and D_j.

We next determine the site-adjustment order. Fig. 5(b) shows that D_3 traverses $S_{3,5}$, $S_{2,3}$ and $S_{3,4}$ serially; D_4 traverses $S_{4,5}$ and $S_{3,4}$ serially; D_5 traverses $S_{4,5}$ and $S_{3,5}$ serially. We combine these lists together into a site-adjustment order: $S_{4,5} \rightarrow S_{3,5} \rightarrow S_{2,3} \rightarrow S_{3,4}$, where the order of these cross-contamination sites maintains the same as in their original lists.

We synchronize the washing steps with functional droplet transportation for the above four sites following this site-adjustment order. For the first site $S_{4,5}$ in the site-adjustment order, we first generate the route of wash droplet $W_{4,5}$ to clean the cross-contamination site $S_{4,5}$, as shown in Fig. 5(c). The route of $W_{4,5}$ consists of two sub-routes. The first sub-route connects the wash reservoir (WR) to $S_{4,5}$, and the second sub-route connects $S_{4,5}$ to the waste reservoir (WS). We next adjust the arrival order of $W_{4,5}$, D_4 and D_5 at site $S_{4,5}$. According to Table 1, D_5 is made to arrive after $W_{4,5}$ traverses $S_{4,5}$. Therefore, D_4, $W_{4,5}$ and D_5 traverse $S_{4,5}$ serially at clock cycle 1, 5 and 9, respectively, in order that $W_{4,5}$ cleans the residue left by D_4 at $S_{4,5}$ before D_5 traverses. After the adjustment for $S_{4,5}$, we record the updated transportation timing when D_4 and D_5 traverses $S_{4,5}$. In this manner, while adjusting the arrival order for the next site $S_{3,5}$, we utilize the newly updated transportation timing information from $S_{4,5}$. Other sites in the site-adjustment order, i.e., $S_{3,5}$, $S_{2,3}$, and $S_{3,4}$, are adjusted by repeating the above process.

Table 2: Comparison between methods for the protein assay.

Sub-prob. no.	N_{cs}	T_r	Results for different methods (proposed/baseline 1/baseline 2)	
			N_{wash}	T_{rw} (clock cycles)
...	...	...	...	...
78	1	8	1/1/2	16/18/16
79	0	4	0/0/0	4/4/4
80	3	16	3/3/4	18/38/20
81	5	19	5/5/7	33/55/42
82	4	18	4/4/5	26/71/34
83	3	13	3/3/5	17/51/23
84	1	8	1/1/2	11/19/15
85	4	18	4/4/6	24/58/27
86	0	1	0/0/0	11/11/11
87	3	17	3/3/5	19/46/33
...	...	...	...	...
Total	89	837	89/89/198	1187/1702/1405

Table 2 shows a fragment of sub-problems in the protein assay. The second column of Table 2 shows the number of cross-contamination sites (N_{cs}), i.e., the number of sites where two functional droplet routes intersect with each other, in each sub-problem. The number of cross-contamination sites can be used to evaluate the likelihood of cross-contamination for a set of functional-droplet routes in a sub-problem. The value $N_{cs} = 0$ denotes the fact that functional-droplet routes obtained are vertex-disjoint, i.e., these routes do not share any cell in the microfluidic array. The third column shows the maximum droplet-transportation time for all nets without wash steps in each sub-problem (T_r).

In Table 2, for each sub-problem, we utilize the proposed wash-operation synchronization method and two baseline methods to completely avoid cross-contamination during the routing of different functional droplets. We compare the proposed method with the two baseline methods in terms of the number of consumed wash droplets (N_{wash}), and the maximum droplet-transportation time for all the nets with wash steps (T_{rw}). Here we assume that one wash droplet is used to clean a cross-contamination site.

Here we analyze the results for Sub-problem 82 in Table 2. For this sub-problem, the proposed method utilizes four wash droplets to clean the four cross-contamination sites. Since wash-droplet routing is synchronized with functional-droplet routing, the maximum droplet-transportation time with wash steps (T_{rw}) is slightly higher than that without wash steps (T_r). Baseline method 1 also utilizes four wash droplets. However, since wash steps are inserted between successive functional-droplet routing steps, the maximum droplet-transportation time with wash steps (T_{rw}) is much higher than that using the proposed method. Baseline method 2 consumes more wash droplets, since one wash droplet has to be appended to each functional droplet. Although this method achieves lower maximum droplet-transportation time than baseline method 1, it is still higher than the proposed method. Similar results are obtained for other sub-problems where cross-contamination sites exist ($N_{cs} > 0$).

We analyze the influence of the number of wash droplets per one cross-contamination site to the maximum droplet-transportation time with wash steps (T_{rw}). Fig. 6 compares the maximum droplet-transportation time with wash steps (T_{rw}) of all the sub-problems for the proposed method with Baseline method 1, when different number of wash droplets is used to clean one

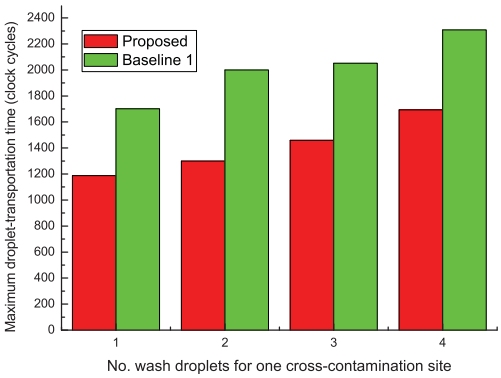

Figure 6: Maximum droplet-transportation time with wash steps (T_{rw}) comparison for the proposed method with Baseline method 1 of the protein assay, when different number of wash droplets is used to clean one cross-contamination site.

Table 3: Comparison of the proposed method with the droplet-routing method in [17] for the protein assay.

Array size	Simulation results (proposed/ [17]/ *diff.*)	
	N_{cs} (N_{wash})	T_{rw} (clock cycles)
8×8	93/47/**46**	1242/1622/**380**
10×10	89/28/**61**	1187/1478/**291**
12×12	85/23/**62**	1244/1391/**147**

cross-contamination site. When the number of wash droplets per one cross-contamination site increases, for both the proposed method and Baseline method 1, T_{rw} also increases, since more time will be spent to route multiple wash droplets to clean one cross-contamination site.

5.2 Comparison with [17]

Finally, we compare the proposed method with [17] for different sizes of the microfluidic array. The parameters N_{cs}, N_{wash}, and T_{rw} in Table 3 are the sum of those for all the sub-problems in a benchmark. The value of *diff.* represents the difference between the proposed method and [17] for each parameter.

Since the droplet-routing method in [17] utilizes disjoint droplet routes to minimize the likelihood of cross-contamination between functional droplets, the number of cross-contamination sites (N_{cs}) is significantly less in [17], and the number of wash droplets (N_{wash}) is also less. Moreover, because the proposed method synchronizes the wash steps with the functional-droplet routing steps, T_{rw} for the proposed method is much lower than that of [17].

For the droplet-routing method in [17], as the size of the microfluidic array decreases, there are fewer free available cells for droplet routing. Thus it is more difficult to obtain disjoint droplet routes, and the number of cross-contamination sites increases. Table 3 shows that the difference in N_{cs} between the proposed method and [17] becomes less significant when the size of the microfluidic array decreases. As a result, in [17], more wash steps have to be inserted between successive functional-droplet routing steps, which leads to increased T_{rw}. Table 3 shows that the difference in T_{rw} between the proposed method and [17] is considerable for smaller microfluidic arrays.

In summary, the simulation results show that compared to [17], the proposed method is more effective for reducing droplet-transportation time without any cross-contamination. While it requires more wash droplets in comparison to [17], this difference is less significant when smaller arrays are considered for low-cost, disposable chips.

6. CONCLUSIONS

We have presented a wash-operation synchronization method that integrates washing steps into functional droplet-routing steps to avoid cross-contamination in a target bioassay. By carefully adjusting the arrival orders of wash droplets and functional droplets at cross-contamination sites, we synchronize the routing of wash droplets with functional droplet-routing steps, thereby reducing droplet-transportation time. A real-life bioassay has been used to evaluate the effectiveness of the proposed method and compare it with [17].

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