A New Design Technique for Droplet Movement and Mixing in Three-Dimensional Digital Microfluidic Biochip

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Abstract

The term 'Lab-on-a-Chip (LOC)' is used to indicate the biochip that can perform different biological and biomedical experiments. The droplet based digital microfluidic biochips (DMFBs) follow the principle of electro-wetting on dielectric (EWOD) for droplet actuation in a digital manner. DMFBs amalgamate electronics with biology and chemistry, and provide a new technology which is highly reconfigurable and scalable. DMFBs oversee the liquid samples and reagents as discrete droplets. As the mixing procedure among all other modular operations is particularly important, the result of the bioassay operation is almost influenced by the mixing operation. Good mixing of appropriate sample and reagent yields authentic result. Here we have proposed a new design technique using 3D square electrode array maintaining all the constraints required to ensure safe droplet routing and other modular operations. Improvement of the mixing operations and incorporating parallel assay operations are the key challenge. Parallelism reduces the assay completion time. We use AC EWOD principles to get the vertical movement of the droplet from bottom plate to upper plate and vice versa, whereas DC voltage is applied to manage the horizontal movement. We can also design the chip through only AC voltage where high frequency is used to droplet movement through 2D plane and low frequency is used for droplet lifting vertically.

Keywords: Lab-on-a-chip (LOC); AC- EWOD; Multiplexed assay operation; Parallelism.

1. Introduction

Now-a-days the droplet elongation and vibration on a solid has drawn the attraction of various research activities due to its importance in different wings of engineering applications, such as digital microfluidics [1], optofluidic optical attenuators and liquid reflective displays, etc. Among the various ways to control the droplet routing, the electrowetting is more convenient because it incorporates the surface wetting property under external electric field. Electrowetting originates from the electrical force concentrated at the three-phase (solid, liquid, and vapour) contact line (TCL). The EW-induced contact angle variation can be described by the Young-Lippmann equation [2]. Electrowetting uses DC voltages to control the interfacial energy between the liquid (biological/biochemical samples or reagents) and the solid substrate (electrodes) so that liquid droplet can move through the dielectric electrodes (Fig.1). Now, when we apply a DC voltage V between the electrode and the droplet, charge is accumulated in the droplet as well as in the

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electrode. As a result, electrostatic energy is stored in the droplet and hence the interfacial tension between the solid and the liquid is decreased yielding the change of droplet shape.

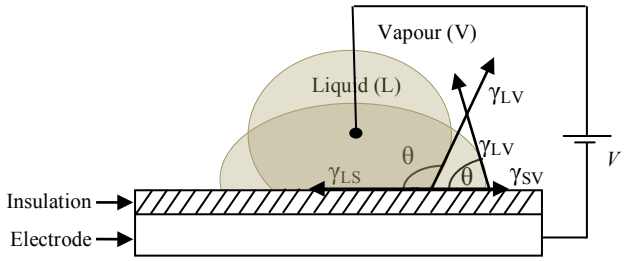


Fig. 1. Electrowetting-on-dielectric (EWOD) effect. Initially the droplet is at rest on the hydrophobic insulated surface. After applying DC voltage V, the interfacial tension of solid-liquid is decreased and the surface area is increases. Generally, the voltages are in the range of 20 V to 80 V. The change in contact angle is inversely proportional to the applied voltage.

When the voltage is applied to the adjacent electrodes, the droplet will tend to increase its contact area, hence the droplet movements occurs [1]. So, by activating and deactivating many electrodes simultaneously through the chip array, the droplet transportation, splitting, mixing can easily be controlled. Like DC, EWOD can use AC electric field also. At very high frequency AC voltage can be used as DC voltage [3]. It is supposed to get a good mixing if we use 3D chip using AC frequency.

2. Design Specification

Water droplet dispensed in silicon oil subject to sinusoidal AC field elongated along the line of force during first half cycle. In the next half cycle, the surface tension pulls the droplet back to its spherical shape. As the scenario is changed because of 3D design, we have to move the droplet through AC frequency regulation. If we regulate the frequency in 44Hz then the droplet size increased to 2.5 mm [3], then it touches the upper cell in the upper conducting plate. Immediately we activate the upper cell and the droplet moves from bottom cell to upper cell. Here we must free the cell where we intend to move the droplet from bottom. Care must be taken for the situation where no droplets are direct or indirect neighbours to the particular cell said above.

A. Fluidic constraints

The restrictions for fluid flow through the chip array are described here. Care should be taken for avoiding unintentional mixing, splitting of the droplets. We can subdivide the fluidic constraints into two parts, viz. (i) horizontal fluidic constraints and (ii) vertical fluidic constraints.

(i) *Horizontal Fluidic Constraint:* Depending upon the positions of the droplets with respect to time horizontal fluidic constraints can be classified into two parts, (a) static constraints, (b) dynamic constraints. There are four rules which demonstrate this type of the constraints and the supporting figure is given below (Fig. 2). Let $D_i\,(X_i(t), Y_i(t))$ and $D_j\,(X_j(t), Y_j(t))$ are two independent droplets used in the chip array at time t.

Static constraints: $|X_i(t) - X_j(t)| \geq 2$, or $|Y_i(t) - Y_j(t)| \geq 2$, i.e. at time t, difference between two droplets should be at least 2 (that means two electrodes).

Dynamic constraints: $|X_i(t+1) - X_j(t+1)| \geq 2$, or $|Y_i(t+1) - Y_j(t+1)| \geq 2$, i.e. the new locations at time $(t+1)$ of the any two droplets should not be adjacent.

$|X_i(t+1) - X_j(t)| \geq 2$, or $|Y_i(t+1) - Y_j(t)| \geq 2$, i.e. the new location of droplet D_i cannot be adjacent to the old location of droplet D_j.

$|X_i(t) - X_j(t+1)| \geq 2$ or $|Y_i(t) - Y_j(t+1)| \geq 2$, i.e. the new location of droplet D_j cannot be adjacent to the old location of droplet D_i.

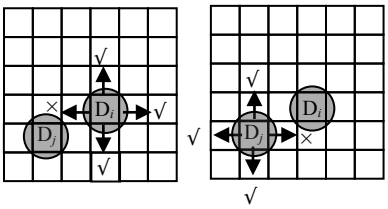


Fig. 2. A demonstration of how fluidic constraint rules are met. "√" refers to allowable movement and "×" refers to not allowable movement.

(ii) Vertical Fluidic Constraint: If we regulate the AC frequency acted upon an electrode on which the droplet is placed in 44Hz then the droplet vertically increased to 2.5 mm [3], and it touches the upper cell in the upper conducting plate. If we immediately activate and deactivate the upper cell and bottom cell respectively the droplet moves from bottom to upper cell. Here we must free the cell (from the other droplet) where we intend to move the droplet from bottom. Care must be taken for the situation where no droplets are on direct or indirect neighbours to the particular cell said above. Depending on these we can formulate the vertical fluidic constraints as follows.

If we lift up a droplet from bottom to top then the assigned position of the top array must be free from other droplet and at the same time all direct and indirect neighbours (total 8 cells) of the targeted electrode cannot contain other droplet, otherwise an unwanted mixing may happen. The above criteria must be satisfied for downward movement also. So it can be concluded that to move a single droplet safely in vertical direction total 18 electrodes are left free in a whole 3-D array (total 9 for upper array and another 9 for lower array).

Fig. 3 demonstrates the vertical movement of a droplet routing in a 3D chip. Suppose a droplet is at (5, 4) position in lower level and its destination will be at (3, 4) of the top level. So at first it starts routing from its initial position and horizontally moves toward the cell (3, 4) of the bottom array (the cell which is just beneath of (3, 4) of the top array). After reaching at (3, 4) of bottom plate, we regulate the frequency from high to low, as a result the vibration in the droplet due to AC frequency changes its shape and the droplet vertically increases to a certain value. As per our previous assumption, we set the gap between the two plates near about 2.5 mm. When the droplet touches the upper cell, we immediately activate the cell (3, 4) in the upper plate with DC voltage and deactivate the bottom cell. As a result, the droplet moves to its destination.

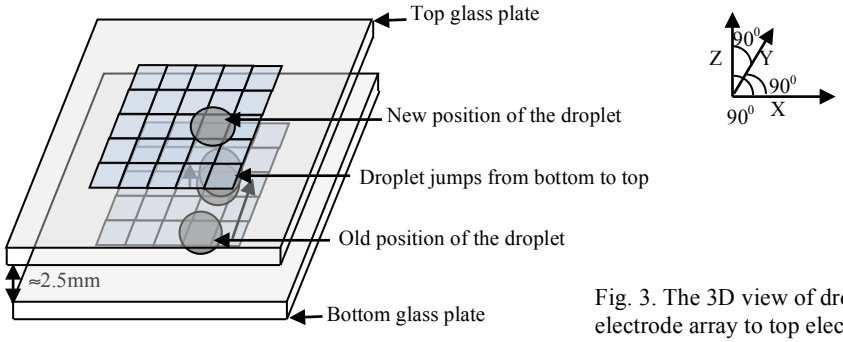


Fig. 3. The 3D view of droplet routing from bottom electrode array to top electrode array.

We cannot move the droplets from top to bottom and bottom to top at the same time whose *X*- and *Y*- coordinates are same but only the *Z*-coordinates are different.

B. Timing Constraint

Timing constraint is the upper limit for droplet transportation time between two modules. It must be ensured that the droplet routing delay does not exceed beyond a particular value, say 10% of a time slot used in the scheduling, such that the droplet movement is completed under a particular time limit.

C. Electrode Constraints

If a droplet is located at electrode (*X, Y*) at time *t*, then this electrode (*X, Y*) must be activated. There are total four electrodes direct and total four electrodes diagonally adjacent to the activated electrode. These electrodes must be deactivated. While activating some electrodes simultaneously, there should not be any interference between the electrodes.

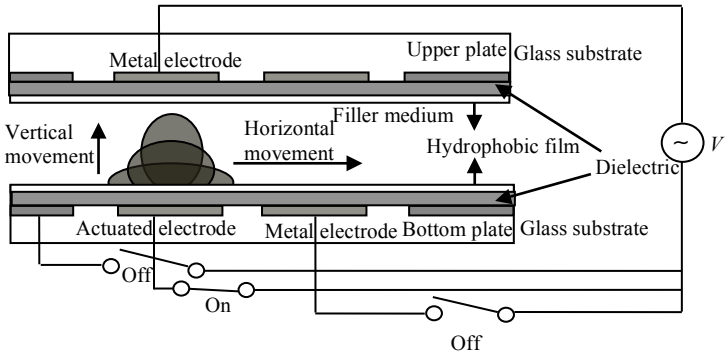
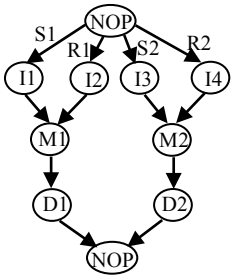


Fig. 4. The cross sectional 3D view of droplet lifting from bottom electrode to top electrode.

To compare the schedule length (in terms of time) based on colorimetric enzymatic reactions we take a real life experiment of multiplexed bioassay operation consist of glucose assay and lactate assay [4]. We take a 15×15 2D square electrode array and a 15×15 3D square electrode array and calculate the time elapsed in both the scenarios. The scheduling graph is shown in Fig. 5.



I*i* : Dispensing operation
M*i* : Mixing operation
D*i* : Detection operation

Fig. 5. Sequencing graph model for multiplexed bioassay. S1, S2 are samples, R1, R2 are reagents, M1 to M2 are mixing operations and D1 to D4 are detection operations, NOP is No Operation.

We model a biochemical application using a sequencing graph. The graph *G* (*V*, *E*) is directed, acyclic and polar (i.e., there is a source node, which is a node that has no predecessors and a sink node that has no successors). Each node *V* represents any one of the operations like dispensing, detection, mixing etc. An edge *e* (*i*, *j*) indicates that the operation O*i* is the input of operation O*j*. In Fig. 5 there is an example of a scheduling graph with eight operations, O1 through O8. Operation O1 to O4 are referred as dispensing operation indicating I1, I2, I3, I4, two mixing operations O5 and O6 are as M1, M2, and finally two detection operation O7, O8 indicating D1 and D2.Thus, O1 is assigned for dispensing from the input port S1 and likely O2 to R1, O3 to S2, O4 to R2. The scheduling is represented by the Gantt chart where we represent the droplet routing, mixing and detection phases as rectangles (Fig. 6). The length of the rectangles represents the corresponding operation's execution time. In case the first Gantt chart, operation O1 and O2 work in parallel, that means firstly S1 and R1 start their routing towards mixer zone. When S1 and R1 are entered in the mixer zone and initiate the mixing operation O5 then operation O3 and O4 are getting started. After completion of operation O5 (first mixing operation at M1), S2 and R2 are entered in the mixer zone. Then operation O6 (second mixing operation at M2) starts and at the same time detection operation D1 for the first mixed droplet is started. Beyond of second mixing operation, the final O8 is performed (detection operation D2 for the second mixed droplet). The second Gantt chart is used

for 3D. In 3D parallelism can be incorporated in an efficient way with the help of upper and lower zone. We can start operations O1, O2, O3 and O4 in parallel that is S1, R1, S2 and R2 initiate routing from the input port towards their corresponding mixer at a time (S1 and R1 input ports are kept in upper zone, S2 and R2 are kept in lower zone). In case of 3D it is advantageous for us that we can create two mixer zones in two levels (upper and lower). So it is clear that the whole arrangement in 3D can support to perform the mixing operations O5 and O6 simultaneously as well as dispensing (S1 and R1 are getting mixed in upper zone, and at the same time S2 and R2 are getting mixed in lower zone). Completion of mixing leads other two operations O7 and O8, in this way both mixed droplets are ready for detection operation at a time as indicated in the Fig. 6. It is clear from the figure that if we use 3D array it is more time efficient, though the routing time in 3D array is greater than the routing time in 2D array, but we can run parallel assay operation in 3D electrode.

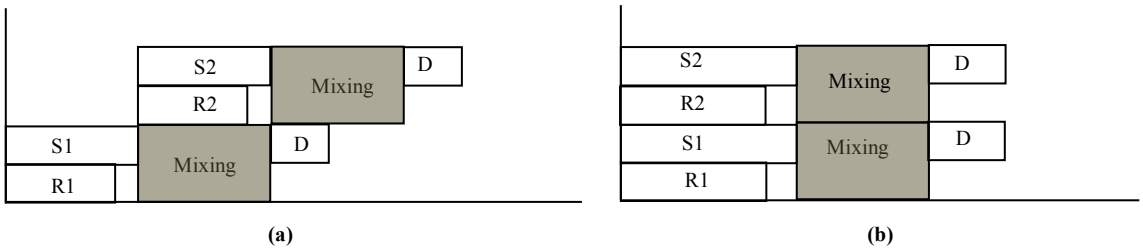


Fig. 6. Gantt chart for scheduling the sequencing graph in Fig. 5. (a) for 2D electrode array (b) for 3D electrode array.

3. Conclusion

This paper introduces a new design technique of digital microfluidic biochip. This new approach is supposed to be a better alternative over conventional 2D electrode array because of incorporating parallelism. Barring this in any assay operation mixing procedure is the most important operation, in case of 3D electrode array, due to the vertical movement of droplet the operation mixing procedure may lead to betterment in case of considering time factor. Beyond the above factors the proper placement of AC electrodes and pin constrained chip design may lead to betterment in assay operation and also minimises the total cost in terms of power. The pin constrained chip design and placement of electrodes on which we want to apply AC voltage such that the total cost in terms of power is minimized may be planed.

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