Synopsis for Project on

Droplet Routing Simulation in Microfluidic Biochips

By

Dipak Kumar Behera

(03CS3018)

Under the guidance of

Prof. Indranil Sen Gupta

Professor
Computer Science & Engineering Department
Indian Institute of Technology Kharagpur
WB, India 721302

1. Introduction

In recent years microfluidics based biochips have become popular for biochemical analysis. These miniaturized microfluidics based biochips can perform enzymatic analysis (e.g. glucose, lactate, and pyruvate assays of human physiological fluids like saliva, urine etc.), massive parallel DNA analysis, automated drug discovery and toxicity monitoring. These biochips can be termed as lab-on-a-chip as it replaces highly repetitive laboratory tasks by replacing cumbrous lab equipments with composite micro-system. The advantage of such biochips over huge and heavy systems is that they provide design flexibility, higher sensitivity and are of smaller size and lower cost. They enable the control of micro- or nano-liters of fluids, thus reducing sample size, reagents volume and power consumption.

There are two techniques by which fluid flow in the microfluidic biochips can be controlled. One is continuous fluid flow carried out by using micro-pumps, micro-valves and micro-channels. The other one, an efficient approach, is to manipulate liquids as discrete droplets. The droplet based technique is referred as “digital microfluidics”. In this approach, each droplet is controlled independently and each cell in the microfluidic array has the same structure. This technique is advantageous over the continuous flow systems because it provides dynamic re-configurability. During the execution of a bioassay a set of cells can be reconfigured dynamically to change their functional behavior.

In [3] the architectural level synthesis is described. The architectural synthesis is similar to a structural RTL model in electronic CAD. First, the behavioral model for a set of bioassays protocol is manually created. Then the behavioral description is mapped to a microfluidic biochip and an optimized schedule for bioassay operations, the binding of assay operations to resources is generated. The geometrical level synthesis, the layout of the biochip consisting of the configuration of the microfluidic array, locations of reservoirs and dispensing ports, and other geometric details is discussed in [4].

The droplet routing is an important job in biochip physical design. Droplet routing problem is to find out droplet paths between modules, and between modules and on-chip reservoirs. Because of the dynamic re-configurability of modules it is highly likely that

two droplet routes will share cells on the microfluidic array at different time intervals. So, the droplet routing in microfluidic biochip is different than VLSI wire routing.

The project aims at the development of a simulation tool for droplet routing of a clinical diagnostic procedure, known as multiplexed *in-vitro* diagnostic on human physiological fluids. Given, the scheduling of different bioassay operations and physical lay out information, the droplet routes are found using the procedure described in later sections and then the movement of droplets between different modules at different time intervals is simulated using a tool developed in Java language.

2. Digital Microfluidic Biochip

The microfluidic biochips are based on the manipulation of nanoliter droplets on a two-dimensional electrode array using the principle of electrowetting [2]. Electrowetting refers to the modulation of the interfacial tension between a conductive fluid and a solid electrode by applying an electric field between them. 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. It is moved by applying a control voltage to an electrode adjacent to the droplet and, at the same time, deactivating the electrode just under the droplet. This electronic method of wettability control creates interfacial tension gradients that move the droplets to the charged electrode. Using the electrowetting phenomenon, droplets can be moved to any location on a two-dimensional array. The basic cell of a digital microfluidics-based biochip is shown in Figure 1(a). Figure 1(b) shows a fabricated microfluidic array.

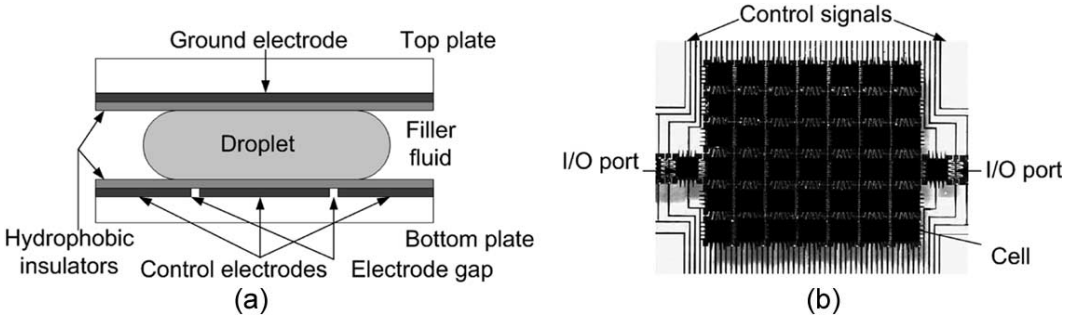


Figure – 1

By varying the patterns of control voltage activation, many fluid-handling operations such as droplet merging, splitting, mixing, and dispensing can be executed in a similar manner. For example, mixing can be performed by routing two droplets to the same location and then turning them about some pivot points. The digital microfluidic platform offers the additional advantage of flexibility, referred to as reconfigurability, since fluidic operations can be performed anywhere on the array. Droplet routes and operation scheduling result are programmed into a microcontroller that drives electrodes in the array. In addition to electrodes, optical detectors such as LEDs and photodiodes are also integrated in microfluidic arrays to monitor colorimetric bioassays.

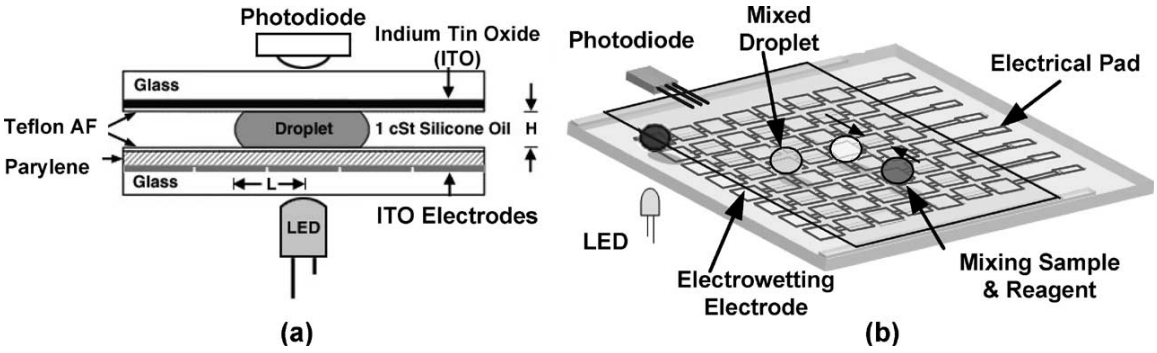


Figure – 2: Digital microfluidics-based biochips used in colorimetric enzyme-kinetic assay [6]

In the microfluidic biochips we have several modules like mixers, diluters, detectors, splitters, waste reservoirs, dispenser etc. These modules can be dynamically formed by activating the corresponding control electrodes during run-time. In this sense, the microfluidic modules can be viewed as virtual devices. We can map the microfluidic assay operations to available microfluidic modules, and then use architectural level synthesis techniques to determine a schedule of sets of bioassays subject to precedence constraints imposed by the corresponding assay protocols [3]. The locations of the modules on the microfluidic array are then determined by the placement algorithms [4].

3. Problem Formulation

3.1. Objective function

The objective of routing problem in microfluidic chips is to find out the path from one module to the other using minimum possible basic cells. To accommodate fault tolerance, i.e. when a primary cell fails to perform bioassay, spare cells are used as primary cells to complete the assay operations. So if the number of cells used during routing is minimized (i.e., droplet route length is minimized) we can be left with more spare cells to accommodate fault tolerance. This is very important in safety critical systems, which are governed by biochips because these types of systems require high fault tolerance.

For routing purpose we require the net informations. A net is defined as the droplet route between pins of different modules. The fluidic ports on the boundary of each module represent pins of that module. The pin assignment is done during the placement phase. So once we get the information about nets we can apply the routing algorithm to find out the droplet routes. In the case of digital microfluidic biochips we can model nets as 2-pin nets or 3-pin nets. A fluidic route on which a single droplet is transported between pins of different modules can be modeled as 2-pin nets. To allow droplet mixing simultaneously during their transport, which is preferable for efficient assay operations, we need to model such fluidic routes using 3- pin nets, instead of two individual 2-pin nets.

3.2. Fluidic Constraints

The accidental mixing of droplets during transportation is avoided except when the two droplets are required merge during mixing operations. So it is always required to keep a safe distance between any two droplets on the chip. Also, during routing of droplets it should always be ascertained that there is no conflict between droplet routes and assay operations. Thus, droplet routing is needed to be isolated from active modules. For the isolation from modules, each module is associated with a segregation region which is wrapped around the functional regions of the modules.

We define three fluidic constraints rules to avoid interaction of multiple droplet routes which intersect or overlap with each other. The microfluidic array is represented by two dimensional coordinates (X, Y). Let $Xi(t)$ and $Yi(t)$ denote the location of droplet, Di at

time *t*. Suppose we have two droplets *Di* and *Dj* initially at time *t*. To avoid mixing of these two droplets they must not be located adjacent or diagonally adjacent to each other. Therefore at time *t*, We must ensure that either $|Xi(t) - Xj(t)| \geq 2$ or $|Yi(t) - Yj(t)| \geq 2$ for these two droplets.

For the well defined locations of the droplets Di and Dj at time t+1 we define these three rules.

Rule #1: $|Xi(t+1) - Xj(t+1)| \geq 2$ or $|Yi(t+1) - Yj(t+1)| \geq 2$, i.e., their new locations are not adjacent to each other.

Following figure shows the violation of Rule #1.

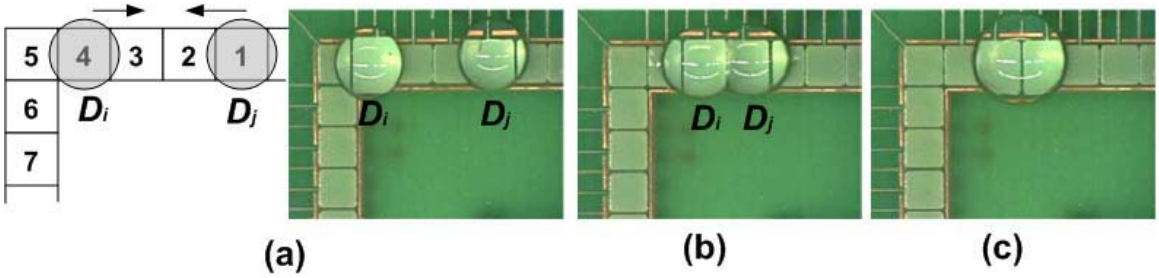


Figure 3: (a) Verification of Rule #1: droplets begin on electrodes 1 and 4; (b) Electrodes 2 and 3 are activated, and 1 and 4 deactivated; (c) Merged droplet.

Rule #2: $|Xi(t+1) - Xj(t)| \geq 2$ or $|Yi(t+1) - Yj(t)| \geq 2$, i.e., the activated cell for droplet *Di* cannot be adjacent to *Dj*. Otherwise, there is more than one activated neighboring cell for *Dj*, which may leads to errant fluidic operation.

Rule #3: $|Xi(t) - Xj(t+1)| \geq 2$ or $|Yi(t) - Yj(t+1)| \geq 2$. Note that Rule #1 can be considered as the static fluidic constraint, whereas Rule #2 and Rule #3 are dynamic fluidic constraints.

Following figure shows violation of Rule #3 is violated for droplet *Di* (i.e., it is directly adjacent to electrode 3 and also diagonally adjacent to electrode 6).

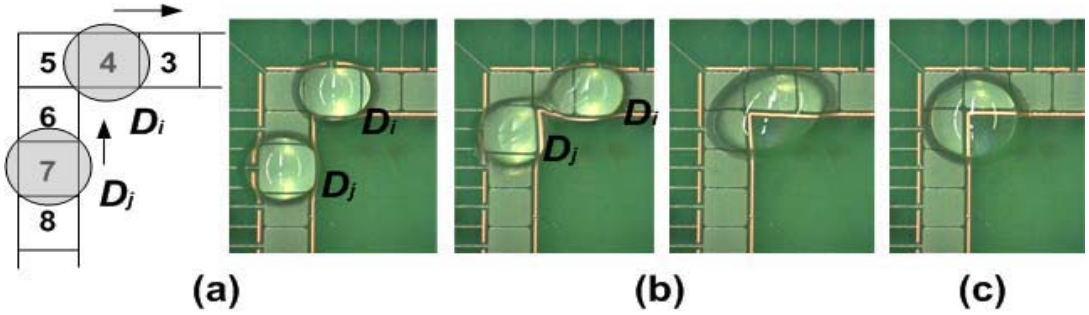


Figure 4: (a) Experimental verification of Rule #3: droplets begin on electrodes 4 and 7; (b) Electrodes 3 and 6 are activated, and 4 and 7 deactivated; (c) Merged droplet.

Moreover, these fluidic constraint rules are not only used for rule checking, but they can also provide guidelines to modify droplet motion (e.g., force some droplets to remain stationary in a time-slot) to avoid constraint violation if necessary; the details of such a strategy are discussed in the section 4.3.

3.3 Timing Constraints.

There is one more important constraint on droplet routing. This constraint is about upper limit on droplet transportation time between two modules. In [3], which describes about architectural level synthesis of microfluidic biochip, it is assumed that since the droplet movement is very fast compared to assay operation (mixing, detection, etc.) times the droplet routing time is not considered while computing a scheduling for assay operations.
So 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. Otherwise, the schedule obtained would no longer be valid. This timing constraint is similar to the interconnect delay constraints in VLSI routing that require each wire net (or critical path) to meet its timing budget. Note that since a droplet may be held at a location in some time slots during its route, the delay for each droplet route is not identical to the route length. The delay for a droplet route therefore consists of the transport time as well as the idle time.

3.4. Problem decomposition

Digital microfluidics based biochips are dynamically reconfigurable. So during the module placement phase a series of 2-D placement configurations are obtained in different time spans instead of a single 2-D placement in classical VLSI design [4].
In this way, the droplet routing problem is divided into a series of sub-problems. In each sub-problem, the nets to be routed between different modules are determined first. Only the microfluidic modules that are active during this time interval are considered as obstacles in droplet routing. Next we attempt to find suitable routes for these nets. These sub-problems are addressed sequentially to obtain a complete solution for droplet routing.

4. Routing Method

The inputs to the algorithm are a list of nets to be routed in each sub-problem as well as constraints imposed by the designer. The droplet routing algorithm consists of two basic stages.

4.1. Phase I: *M*-shortest routes

In this phase, *M* alternative routes for each net are generated. The maze routing algorithm can be applied to find out the routes.

4.1.1. Two-pin nets.

The shortest route problem for 2-pin nets is equivalent to the single-pair shortest path problem.

4.1.2. Three-pin nets

We use 3-pin nets to model the routes along which two droplets are transported towards a microfluidic module (e.g., a mixer); the droplets can mix together during their transportation. The shortest-route problem for such nets is equivalent to the Steiner Minimum Tree (SMT) problem.

Out of these M alternative routes those which fail timing delay constraint check (TDCC) are discarded.

4.2. Phase II: random selection

In the second phase of the algorithm, a single route from the *Mi* alternatives for each net i is selected, where $i \in \{1, 2, \ldots N\}$ and N is the number of nets. Note that $M_i \leq M$ since some routes that violate the timing constraint have already been eliminated. A random selection approach is then used to select i_k for each net i, where i_k represents the k-th alternative route for net i, and $k \in \{1, 2, \ldots, M_i\}$. A desirable feature of this random method is that it avoids the net-routing order dependence problem.

The set of selected routes are evaluated on the basis of number of cells used in routing.
The cost function C = number of cells used in routing. Then once again we check the constraint upon selected routes. If it fails fluid constraint rule check (FCRC) (including droplet motion modification discussed in Section 4.3) or TDCC, we assign a large

penalty value *Pt* to this set of routes, so that these routes are not selected. Otherwise, we set *Pt* = 0 for those that satisfy all constraints.

After an adequate number of random selection runs, we select the set of routes with the minimum cost value *C* and *Pt* = 0 as the output of the routing algorithm.

4.3. FCRC and droplet motion modification

Assume that two droplet routes (i.e., *Pi* and *Pj*) have been obtained. To adhere to fluidic constraint rules, we need to check these two droplets *Di* and *Dj* in each time slot. Interestingly, even if a rule violation is found, we can still modify droplet motion (i.e., force a droplet to stay in the current cell instead of moving) to override the violation; see Table 1. If the modification fails (as in last the row of Table 1), the corresponding routing paths are deemed to be infeasible. We can further extend the modification to the case of more than two droplet pathways.

Rule#1	Rule#2	Rule#3	Modification
Pass	Pass	Pass	Not required
Pass	Pass	Fail	D_j stays
Pass	Fail	Pass	D_i stays
Pass	Fail	Fail	N/A*
Fail	Pass	Pass	Droplet with the smaller $DL(P)$ stays
Fail	Pass	Fail	D_j stays
Fail	Fail	Pass	D_i stays
Fail	Fail	Fail	Fail

*N/A denotes that this case does not exist.

Table - 1

5. Experimental Evaluation:

The assay protocol for in-vitro diagnostics of human physiological fluids can be modeled as sequencing graph as shown in figure 5. Figure – 6 shows the scheduling of assay operations. Figure – 7 shows the net informations while solving sub problem 5. All these informations are given a priori to evaluate the routing. After solving all the sub problems, routing results are obtained and then the droplet movement is simulated.

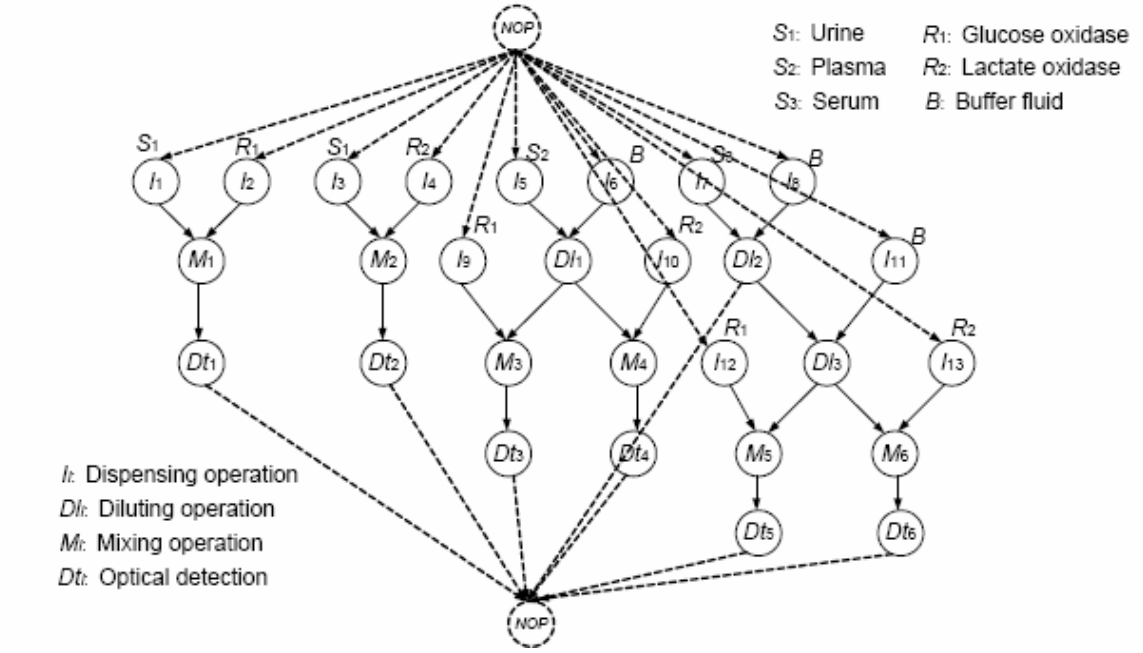


Figure – 5: Sequencing graph model of assay example

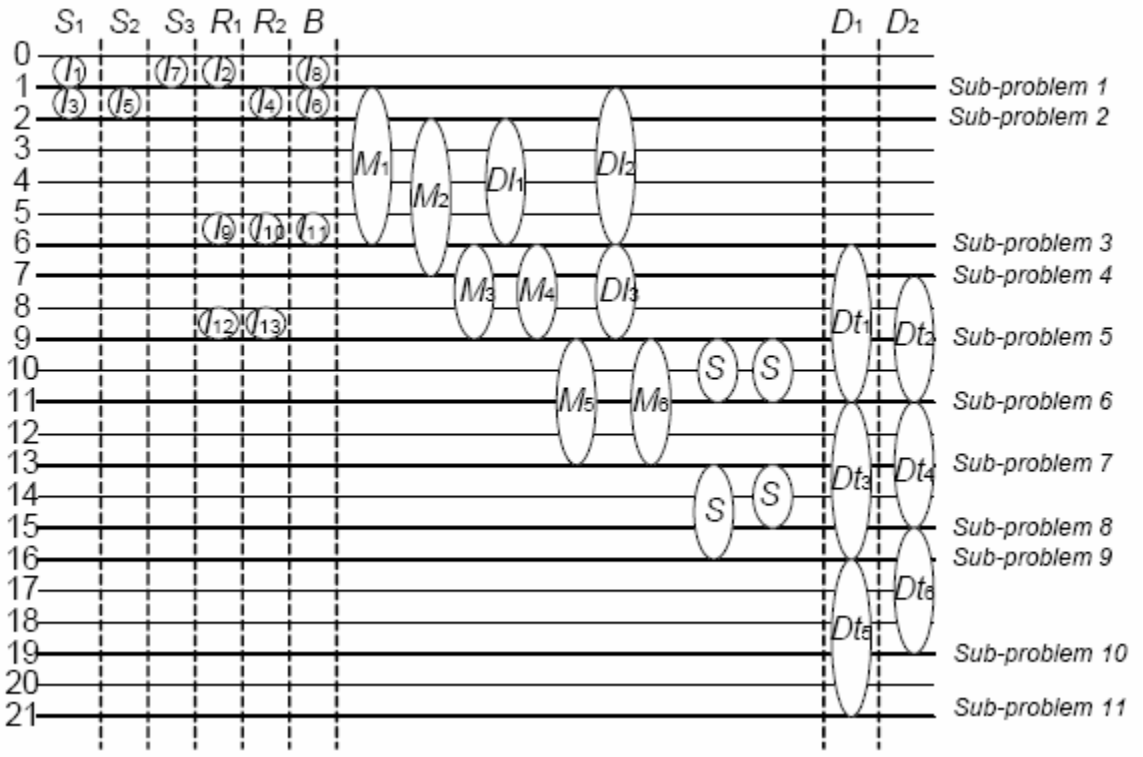
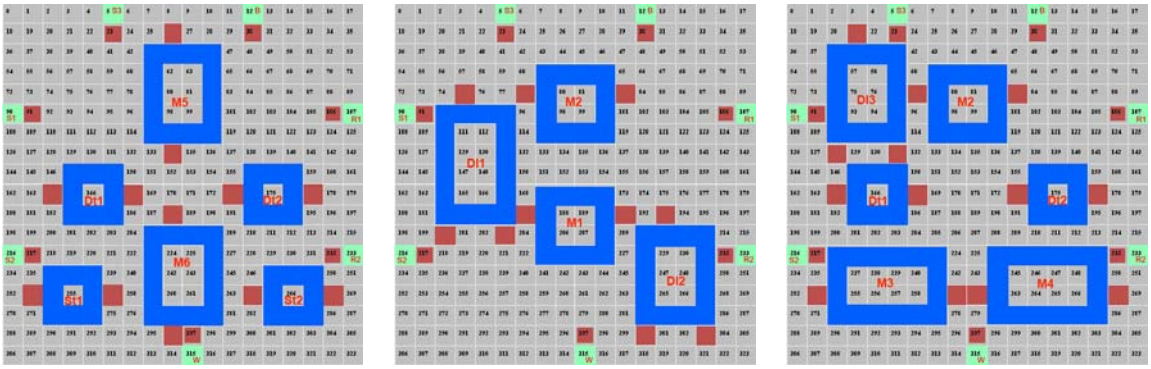


Figure – 6: Schedule obtained via architectural level synthesis and decomposition into sub-problems



(a) (b) (c)

Figure – 7: Module Placement. The microfluidic array size is 16 x 16
(a) For time slots < 6 (b) for time slots 6 – 9 (c) for time slots > 9

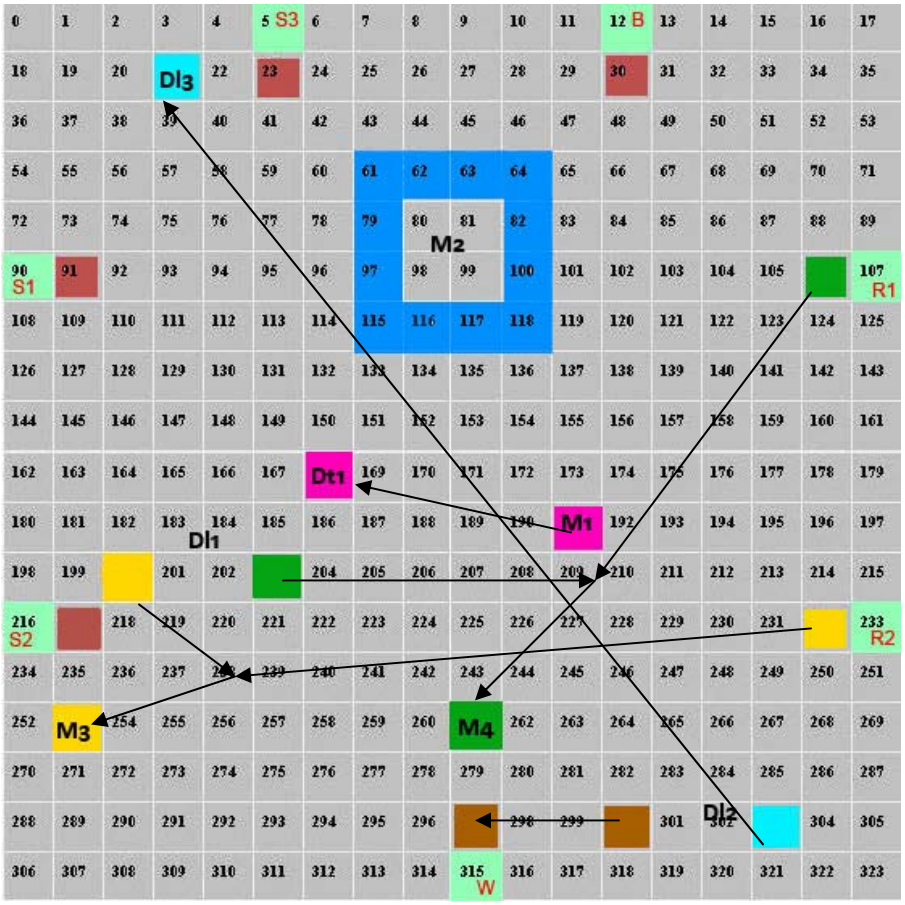


Figure – 8: three 2-pin nets and two 3-pin nets to be routed in solving sub problem 5. Module M2 act as an obstacle while routing because it is active during that time interval.

6. References

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