# Cyber-Physical Integration in Programmable Microfluidic Biochips

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***Abstract*—Microfluidic biochip technology integrates miniaturized components into a chip that can perform traditional biochemical laboratory procedures. Commercial impact is highlighted by the recent acquisition of Advanced Liquid Logic by Illumina Inc., a leader in DNA sequencing and biomolecular analysis. Due to the inherent variability involved in many biochemical processes, uncertainties manifest themselves in many ways in microfluidics. Cyber-physical integration of on-chip sensors permits feedback-driven monitoring in-real time to detect and correct errors, along with other benefits such as adaptive control and dynamic re-synthesis. This paper overviews flow-based and digital (droplet-based) microfluidic biopchips, and discusses the state-of-the-art in microfluidic device fabrication, the interplay between sensor feedback and adaptive control software, and practical experiences relating to biochip cyber-physical integration. It demonstrates the connections between the many fundamental principles of chip design and engineering, and the needs of the biochip community.**

***Keywords*—Biochip, Cyber-Physical System, Error Detection and Recovery, Microfluidics, Sensor.**

## I. Introduction

Microfluidic biochip technologies, which integrate various miniaturized components into a chip for implementing a variety of biochemical operations, have gained increasing popularity recently. Compared to traditional biochemical laboratory procedures, the advantages are many, including smaller instrument size (for portable and point-of-care applications), reduced reagent consumption (for decreased assay cost), reduced sample requirements (important for medical diagnostics), faster reaction kinetics (for quicker operation), enhanced parallelism (for increased throughput), and automation (to eliminate operator errors). Microfluidic biochip technologies have found important applications in many areas such as DNA sequencing, proteomic analysis, clinical diagnostics and drug delivery.

There are two types of microfluidic biochips, namely, flow-based biochips and digital (droplet-based) biochips. The basic idea of flow-based biochips is to utilize micropumps, microvalves and microchannels to control the continuous fluidic flow on the microliter scale. The earliest flow-based microfluidic devices were made using silicon wafers. By leveraging microfabrication tools developed by the semiconductor industry, researchers created a variety of "lab-on-a-chip" or "micro total analysis systems" in silicon. However, silicon has several drawbacks for microfluidic chips, including its optical opaqueness, rigidity and expense. Later, glass became a popular substrate for microfluidics because of its optical transparency and chemical inertness, and many of the highest-performance microfluidic analysis devices continue to be made of glass [1]. However, the rigidity of glass continued to frustrate efforts to integrate valves and pumps in glass microfluidics, and the complex microfabrication equipment required to make silicon and glass microfluidics slowed the spread of the technology. In 1998, George Whitesides' group at Harvard demonstrated "soft lithography," a technique for casting microfluidic devices in silicone rubber [2]. This technique revolutionized the microfluidics field by providing a relatively simple way for virtually any researcher to make microfluidic chips. The flexibility of silicone also made it an ideal material for moving parts in microfluidics, so silicone-based valves and pumps were developed for silicone [3] and glass [4] microfluidic devices. These valves and pumps are controlled pneumatically (using air pressure or vacuum) and can be fabricated by the hundreds in a microfluidic chip; they enable microliter-scale volumes of fluids to be routed on-chip.

In contrast to the flow-based microfluidic biochips, digital microfluidic biochips (DMFBs), where liquids are manipulated as independently controllable discrete droplets, have gained increasing popularity recently in both academia and industry. While in flow-based biochips, continuous fluidic flows are manipulated in DMFBs each droplet can be transported, stored, mixed and reacted in a discrete manner using a set of pre-defined basic operations. A DMFB consists of a 2D electrode array together with some peripheral devices (e.g., dispensing ports). Droplets are controlled by underlying electrode actuation to generate electrowetting force, which can move around the entire 2D array to perform fundamental operations (e.g., dilute, mix). Note that these operations can be performed in a reconfigurable fashion, i.e., different operations can be performed at the same location at different time steps.

## II. The Need For Programmability In Biochips

### A. Flow-Based Biochips

The evolution of microfluidics—from Terry's simple gas chromatograph chip [5] to modern devices that contain complex networks of channels and hundreds of valves—mirrors the evolution of the integrated circuit from Kilby and Noyce's first IC to modern microprocessors containing billions of transistors. However, while microfluidics and ICs may have evolved similarly, the processes by which these different chips are designed and controlled are dramatically different. First, while modern microprocessors are designed using automated software tools, modern microfluidic chips are still designed by hand. Manual design of microfluidics severely limits the complexity of microfluidic devices and slows the development of new chips for new applications. Second, while microprocessors can run programs, microfluidic chips largely cannot. The programmability of microprocessors enables the same hardware to run an infinite variety of different applications. But most microfluidic chips perform only a single application, and a single chip generally cannot be used for a range of different applications. Thus, currently each new application for microfluidics requires a whole new chip design, created by hand by an expert in the field. This process is remarkably slow and inefficient; it is responsible for keeping important and potentially-lifesaving microfluidic tools out of the hands of users who need them. This paper provides an overview of emerging research that seeks to impart programmability and design automation to the field of microfluidics.

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A crucial first step toward the vision of programmable microfluidics is integrating logic onto the microfluidic chip itself. Currently all of the logic of controlling a microfluidic device is located off-chip, in traditional computers and other electronic hardware. This off-chip hardware adds considerable size, cost, and complexity to microfluidic instruments. If the logic of microfluidic chip control could be integrated into the microfluidic chip, off-chip hardware could be dramatically reduced or even eliminated, thereby making the instrument more suitable for use in resource-limited or point-of-care applications.

One way to integrate the logic of a microfluidic chip on-chip is to use microfluidic valves to control not only fluid but also other valves (thereby functioning like transistors in an IC). By arranging valves in various pneumatic "circuits," Boolean logic gates can be created (for example, two valves in series function as a Boolean AND because air will flow through them only if both valves are open, and two valves in parallel function as a Boolean OR because air will flow if either valve is open). In this manner, complex logic functions can be built by simply arranging on-chip valves in the appropriate "circuit". Figure 1 shows a microfluidic chip containing 128 pneumatic valves that function together as an 8-bit ripple-carry adder [6]. This adder is capable of adding any two 8-bit numbers on-chip using only air pressure and vacuum (and no electricity or off-chip hardware other than a vacuum pump). Recent work has shown that entire microfluidic finite state machines can be built using valves as logical elements [7]. This raises the possibility of future programmable microfluidic chips that both control fluids and run programs using on-chip valves.

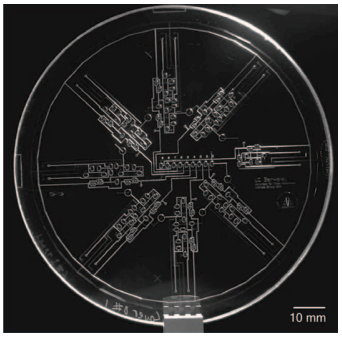


Fig. 1. 128 microfluidic valves arranged in a pneumatic logic "circuit" that can add any two 8-bit numbers.

### B. Digital Biochips

The need for design automation and programmability in digital biochips can be explained similarly. The complexity of digital biochip designs has been steadily increasing recently due to the following reasons. Multiple bio-assays are expected to be performed concurrently, which in turn require more complex resource management. In addition, considerable efforts are needed to address fault tolerance and reliability concerns in the biochip design. Further, cyber-physical integration, which will be described later, demands more design efforts in the sensing and control systems. Together with the tight time-to-market constraint, full-custom design is clearly not suitable to handle biochips with high complexity, which necessitates highly effective and efficient computer-aided design (CAD) techniques.

As mentioned before, each biochemical operation can be performed anywhere within the 2D chip space, which is known as reconfigurability. These mean that one can construct a library of modules to be reused in designing different digital microfluidic biochips. The programmability and reconfigurability naturally lead to a module-library-based methodology, which is similar to the gate-library-based methodology in VLSI design. For example, performing placement and routing among modules in a biochip is in the same spirit as performing placement and routing among gates in a VLSI circuit. Just as CAD has become indispensable in handling large scale and highly complex VLSI circuits, automation is also needed for high complexity digital biochip designs.

Recent advances in biochip CAD include automated architectural level synthesis, physical level optimization (such as fluidic-operation scheduling, module placement and droplet routing), testing and verification. These CAD techniques can significantly alleviate the workload of biochip designers, making them focus more on the development of bio-assay protocols rather than optimization algorithms. They also allow biochip users to more easily adapt to the new technologies in the future. Ultimately, automation techniques will revolutionize the digital biochip design process just as what we have experienced in the VLSI circuit design.

## III. Cyber-Physical Integration

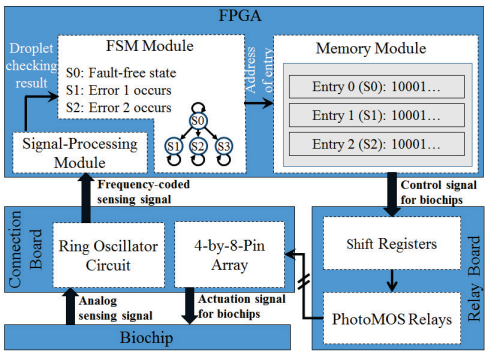


Fig. 2. Schematic of the integrated system setup.

### A. Experimental Demonstration

Due to the inherent variability involved in many biochemical processes, uncertainties manifest themselves in many ways in both of flow-based and digital microfluidic biochip designs. For example, uncertainties might occur due to physical defects, charge trapping and protein fouling. To handle these uncertainties, cyber-physical integration stands out as a promising solution. It deploys on-chip sensors to enable feedback-driven monitoring in-real time to detect and correct errors, along with offering other benefits such as adaptive control and dynamic re-synthesis. This technology can benefit both types of microfluidic biochips. In either case, the sensing system can help identify the potential errors through monitoring the status (in terms of e.g., concentration) of fluidic flows or digital droplets, and subsequently error recovery procedure (such as rolling back and reperforming biochemical operations) can be conducted. As an example demonstration of cyber-physical integration in microfluidic biochips, this paper presents recent advances in the cyber-physical adaptation for digital microfluidic biochips.

Several studies have recently been reported for integrating DMFBs with a control system that can enable real-time recovery from errors during bioassay execution [8], [9], [10], [11]. Among these studies, the work in [9] was the first to present an experimental demonstration of error recovery in cyber-physical DMFBs. However, in this study, a desktop computer is involved in the control system in order to generate the new electrode actuation sequences for recovery. The drawbacks of this approach are: (1) increased complexity of the cyber-physical system; (2) the need for a software resynthesis step, which leads to increased bioassay response time when errors occur.

A computer-in-the-loop control approach has also been used in [12]; a real-time electrode-driving technique was proposed to manipulate multiple droplets. Fault tolerance is achieved by using a

fuzzy-enhanced control system that is able to identify deteriorated electrodes and flexibly adjust droplet routes in real-time. However, the complex inference engine significantly increases system complexity. In [13], the electrode-driving technique was enhanced to increase droplet velocities while elongating the lifetime of the biochip. However, it also involves a desktop computer that controls biochip actuation through an FPGA board according to the feedback from a high-speed camera system.

A dictionary-based and hardware-assisted error-recovery technique has been reported recently [14]. For a target set of likely errors, simulations are used to generate an error dictionary before the experiments are conducted. During simulation, erroneous fluidic operations are considered and the corresponding error recovery plans are determined and then stored as entries in the dictionary. Once an error is detected during the experiment, the control system triggers the corresponding error-recovery plan based on the error dictionary.

The dictionary-based error recovery has been implemented using a finite-state machine (FSM). The control signals for the biochip are determined by the current state of the FSM; an integrated capacitive sensor is used to provide the feedback that indicates whether an error has occurred. If an error is detected, a state transition in the FSM is triggered. The error dictionary stored in the FPGA memory is a precomputed database that links each possible state to the corresponding control signals for the biochip. When the sensing system detects an error, the FSM transitions to the corresponding faulty state and the linked entry in the error dictionary is loaded and sent to the chip for error recovery.

Experimental results have been reported based on a fabricated silicon device and an integrated system. Links to videos are available in [14] to highlight the capability of hardware-based real-time control of multiple droplets, autonomous error detection through capacitance sensing, and the human-intervention-free re-routing of droplets to bypass faulty sites.

The integrated system consists of three parts: a relay board, a connection board, and an FPGA with integrated memory. Figure 2 shows a schematic of the integrated system setup. The relay board is used to synchronously actuate the 32 electrodes in the biochip. The input to the relay board is the low-voltage control signal provided by the FPGA (0-5 V), and the output is the high-voltage actuation voltage for the biochips (40 V, 1 k HZ AC voltage). Four 8-bit serial-in-parallel-out shift registers and 32 photoMOS relays are integrated on the relay board. The shift registers are used to realize serial-to-parallel conversion of the control signal, and the photoMOS relays are used to convert the signal from the low-voltage control signal to the high-voltage actuation signal for the biochip.

The connection board contains two modules: a 4-by-8-pin array and a ring oscillator circuit used for capacitive sensing. The pin array on the back of the connection board can contact the metal pads on the biochip so that the high-voltage actuation signal is transferred from the relay board to the chip. The circuit diagram for capacitive sensing can be found in [11]. The ring oscillator is used to detect the presence or absence of droplets by monitoring the capacitance change between the control and ground electrodes.

The circuitry for cyber-physical error recovery consists of three main modules: (1) the signal-processing module that analyzes the frequency feedback signal from the capacitive sensing circuit and determines if an error has occurred, (2) the memory module that stores the error dictionary, and (3) the FSM module that dynamically adjusts the actuation sequences when an error is reported. Figure 2 illustrates the interconnection between these modules.

The FPGA device used in the experiment was an Altera Cyclone IV with 6.3 Mb embedded memory. Several experimental runs were carried out using the fabricated chip and the control system. A CCD camera was used to capture the video, and images were extracted from the recorded video. Videos of the error-recovery experiments are available online [14]. Droplets were routed as discussed in [14]. The fault-free video shows the initial droplet route. If an error occurs, the droplets cannot reach the checkpoints as planned in the experiment. At that point, the corresponding entry in the error dictionary is activated and the error-recovery solution is triggered.

### *B. The Algorithm: Co-Optimization of Sensor Deployment and Module Placement*

The above workflow demonstrates the effectiveness of sensing-based cyber-physical integration for error detection and recovery in DMFBs. However, it assumes the locations of sensors are fixed which can be treated as blockages in the module placement stage that could impact the overall bioassay completion time in the DMFB design. Minimizing the bioassay completion time is very important because an increase in the time-to-result for a bioassay is detrimental to real-time detection, rapid response and analysis of biochemical samples that degenerate rapidly. To tackle this problem, this section outlines a co-optimization algorithm considering sensor deployment and module placement in DMFB design simultaneously. Based on previous discussion, the co-optimization of sensor deployment and module placement problem for DMFB can be summarized as follows.

Input:

1) A module library: The library contains modules for each type of operation, such as mixing, dilute, and sensing, with different dimension (i.e., width, length, error range, and executing duration).
2) Bioassay protocol: Given an acyclic graph $G = (V, E)$, where the vertex set $V = \{v_1, \ldots, v_m\}$ and the edge set $E = \{e_1, \ldots, e_n\}$ denote the operations and the precedence relations between two operations. The graph $G$ is also called a sequential operation graph.
3) Design specifications: Given the maximum allowable execution time, $T$, the threshold error limit, $T_e$, the size of microfluidic array, $W \times H$ , and the available number of each type of resources as design constraints.

Constraints:

1) Precedence constraint: The precedence relationship exists between two vertices $v_i$ and $v_j$. If $v_i$ is a predecessor of $v_j$ in the sequencing graph, $v_j$ can only start after $v_i$ is finished.
2) Resource constraint: Resources include the dispensing port of the specific regent buffer or sample and the optical detector. Based on the available number of resources, there cannot be more than the available number of each type of resource at the same time. In addition, the optical detector is a special resource because its location is decided at the fabrication stage and is fixed.
3) Storage constraint: After all the operations are scheduled and bound into a placement, the storage unit should be inserted between the precedence $v_i$ and succession $v_j$ to guarantee a space for storing the droplet if the successive operation $v_j$ does not start right after the finish of the precedent operation $v_i$.
4) Reliability constraint: An electrode cannot be activated for more than the limited continuously time period to prevent the charge problem.
5) Sensing constraint: A droplet is required to move to a sensor to check the quality if the error limit of a droplet is larger than the threshold error limit.
6) Fixed-outline constraint: The area occupied by all the modules cannot exceed the dimension of the chip.
7) Nonoverlapping constraint: At any time, no two modules can overlap.

Objective: To synthesize a module placement for DMFBs by scheduling the bio-assay operation, binding assay operation to resources, and creating a 3-D fixed outline layout with optimized biochip assay completion time while considering the sensor deploy-

ment. Figure 3 shows the 3-D placement flow for DMFBs and a corresponding example.

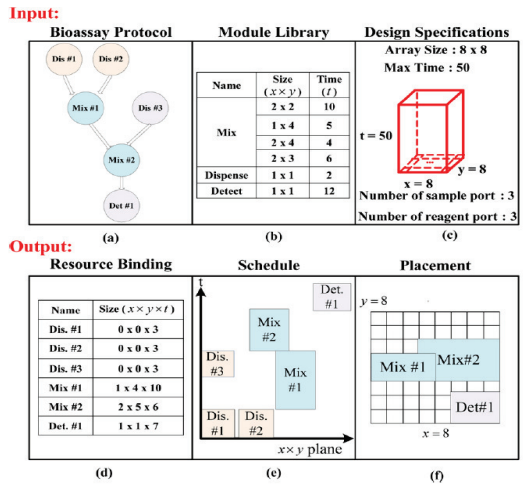


Fig. 3. DMFB placement synthesizing flow. (a) Sequencing graph for a bioassay protocol. (b) Module library. (c) Design specification. (d) Resource binding results. (e) Scheduling of modules. (f) Placement results of modules.

The co-optimization for on-chip sensor and module placement can be solved by the 3D deferred decision making (3D-DDM) framework [15]. After the partitioning of the assay sequencing graph, a deferred decision making is developed to minimize bio-assay completion time by enumerating possible module combinations and merging the combinations into several feasible placements. Moreover, to avoid cross contamination, sensors should not overlap with any module during placement. Therefore, a sensor reuse methodology can reduce the completion time overhead due to washing potential contamination.

### C. Future Design Challenges

In a cyberphysical digital microfluidic system, the monitoring of droplets is vitally important. Researchers often use cameras fixed on the hardware platform to simultaneously monitor multiple operations on the biochip. From the captured images, the volumes and concentrations of the droplets can be determined at each step of the bioassay, and the time required to complete the dilution/mixing processes on the biochip can be measured precisely. However, misleading results concerning the completion of these processes may be derived if only the top view is used for monitoring the fluid-handling operations. Thus, it is essential to monitor cyberphysical microfluidic biochips by optimizing the position of both top and side views simultaneously.

On a cyberphysical microfluidic biochip, we can potentially implement a bioassay with a decision-tree architectures. Decision-tree architectures will enable new functions for a biochip, such as biochemical analysis for unknown analytes. Droplets with an unknown sample can be used as an input to the biochip. Then, the droplet will be sent to a series of checkpoints and the droplet's constituents are checked step by step. Based on the detection result at each checkpoint, the biochip will determine the next operations that will be implemented on the droplet.

In current literature, all droplets are driven by the same voltage, and their corresponding charging times of the electrodes are the same. However, as the "mobility" of droplets vary, a fixed charging time for driving all the droplets may be unnecessarily long for some droplets. The excessive charging time for electrodes may increase the degradation of electrodes. To avoid this problem, a cyberphysical microfluidic biochip may dynamically adjust the voltage and charging time for driving droplets according to their "mobilities". In this way, the reliability and lifetime of biochips can be improved while the power-consumption can be reduced.

## IV. Conclusion

This paper reviews the state-of-the-art flow-based and digital (droplet-based) microfluidic biochip technologies in terms of microfluidic device fabrication, the interplay between sensor feedback and adaptive control software, and practical experiences relating to biochip cyber-physical integration. In particular, due to the inherent variability involved in biochemical processes, uncertainties manifest themselves strongly in microfluidics. This paper describes a cyber-physical integration framework which leverages on-chip sensors for real time feedback-driven monitoring, error detection, error correction, adaptive control, and dynamic re-synthesis. Such a framework has the great potential to significantly mitigate the variational impact due to various uncertainties inherent in many biochemical processes.

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