Design and Test Continuous-flow Microfluidic Biochips

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Glossary

DAPI 4',6-diamidino-2-phenylindole; a fluorescent stain that binds strongly to DNA and serves to marks the nucleus in fluorescence microscopy

DEPC diethyl-pyro-carbonate; used to remove RNA-degrading enzymes (RNAases) from water and laboratory utensils

DMSO dimethyl sulfoxide; organic solvent, readily passes through skin, cryoprotectant in cell culture

EDTA Ethylene-diamine-tetraacetic acid; a chelating (two-pronged) molecule used to sequester most divalent (or trivalent) metal ions, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), copper (Cu^{2+}), or iron (Fe^{2+} / Fe^{3+})

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Introduction

1.1 The rise of the chip

The field of microfluidics has four parents: molecular analysis, biodefence, molecular biology and microelectronics. First came analysis. The distant origins of microfluidics lie in microanalytical methods gas-phase chromatography (GPC), high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE) which, in capillary format, revolutionized chemical analysis. These methods (combined with the power of the laser in optical detection) made it possible to simultaneously achieve high sensitivity and high resolution using very small amounts of sample. With the successes of these microanalytical methods, it seemed obvious to develop new, more compact and more versatile formats for them, and to look for other applications of microscale methods in chemistry and biochemistry. A second, different, motiva

The first key area that inspired the field of microfluidics is molecular analysis.

A second, different, motivation for the development of microfluidic systems came with the realization after the end of the cold war that chemical and biological weapons posed major military and terrorist threats. To counter these threats, the Defense Advanced Research Projects Agency (DARPA) of the US Department of Defense supported a series of programmes in the 1990s aimed at developing field-deployable microfluidic systems designed to serve as detectors for chemical and biological threats. These programmes were the main stimulus for the rapid growth of academic microfluidic technology.

The third motivational force came from the field of molecular biology. The explosion

of genomics in the 1980s, followed by the advent of other areas of microanalysis related to molecular biologies, such as high-throughput DNA sequencing, required analytical methods with much greater throughput, and higher sensitivity and resolution than had previously been contemplated in biology. Microfluidics offered approaches to overcome these problems. The fourth contribution was from microe

The original hope of microfluidics was that photolithography and associated technologies that had been so successful in silicon microelectronics, [The origins and the future of microfluidic]

Microfluidic biochips are revolutionizing the traditional biochemical experiment flow with their high execution efficiency and miniaturized fluid manipulation (**? ? ?**). Devices are built in such a chip to execute specific operations, such as mixing and detection. Fluid samples are transported through microchannels between devices to carry out the protocol of a bioassay. All these functions are performed at the nanoliter level and controlled by a microcontroller without human intervention. The efficiency and reliability of such miniaturized and automated chips endow them with a great potential to improve human life significantly, and the research to bridge them with real-world applications is key to their success.

A flow-based microfluidic biochip is constructed from basic components such as microchannels and microvalves, henceforth named as channels and valves for simplicity. Flow channels are used to transport reaction samples and reagents between different locations. Above flow channels, control channels are built to conduct air pressure to intersections of flow channels and control channels to form valves, as illustrated in Figure 1.1(a), where three valves are constructed at the intersections. These channels are built from elastic materials, so that air pressure in a control channel can block the movement of fluid sample by squeezing the flow channel downwards. Conversely, if the pressure in the control channel is released, the fluid sample can resume its movement. Since the channel width has been miniaturized down to 50 um (**?**) thanks to the advance of manufacturing technology, a huge number of channels and valves can already be integrated into a single biochip to perform large-scale experiments and diagnoses.

With valves as basic controlling components, complex devices can be constructed. For example, mixers can be built using channels and valves to execute mixing operations, which are very common in biochemical applications. The structure of a mixer is shown in Figure 1.1(b), where the three valves at the bottom are actuated alternately

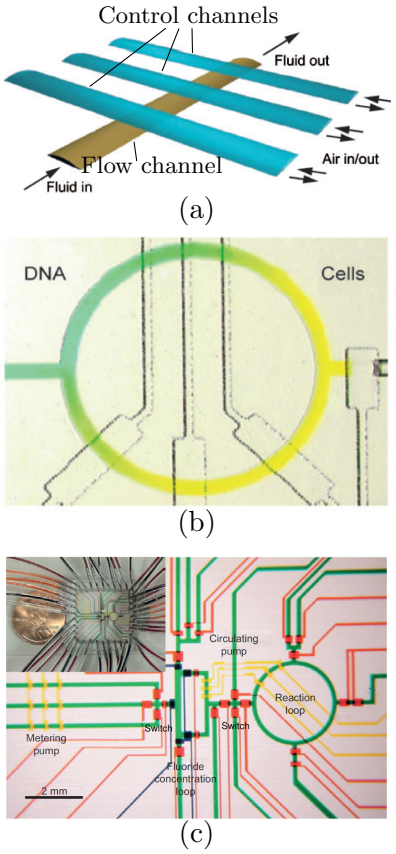

Figure 1.1: Components and structure of flow-based biochips. (a) Valves constructed at intersections of flow/control channels (**?**). (b) Mixer (**?**). (c) A part of a biochip containing a mixer surrounded by a transportation channel network (**?**).

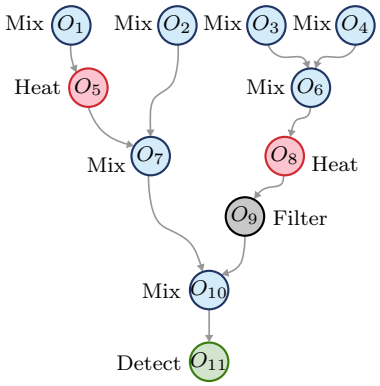

Figure 1.2: Sequencing graph of a bioassay.

by applying and releasing air pressure in the control channels to mix fluid samples and reagents by peristalsis. The execution of a mixing operation in a biochip is demonstrated in a video (**?**). After the mixing operation is completed, the resulting fluid sample can be stored in a dedicated storage unit temporarily.

In a biochip, devices executing specific operations, e.g., mixing and heating, are connected by channels so that intermediate reaction results can be transported between devices for processing. All these operations and transportation are controlled by a microcontroller, which issues instructions in a given order to actuate valves to move fluid samples and execute operations. Figure 1.1(c) shows a mixer (reaction loop) surrounded by flow channels (green), control channels (yellow and red) and valves (yellow and red blocks). These channels and valves together form a network similar to the road transportation system. If fluid channels should cross, valves are built to form a switch, as shown in Figure 1.1(c). At any moment, only two out of the four valves should be opened to direct fluid transportation; the other two valves must be closed to avoid fluid contamination. Consequently, the role of the valves at the intersection of flow channels is similar to that of the traffic lights in the road transportation system, while the open/closed states of the valves are controlled by a microcontroller according to the protocol of the application. The mixer and the channel network in Figure 1.1(c) are implemented into a biochip of the size comparable to that of a coin as shown at the upper left corner, demonstrating the miniaturized integration of microfluidic biochips.

In a biochip, the open/closed states of valves and the transportation of fluid samples are determined according to the biochemical application executed by the biochip. A biochemical application, or *bioassay* henceforth, is usually described with a *sequencing*

graph $\mathcal{G} = (\mathcal{O}, \mathcal{E})$, such as in Figure 1.2, where $\mathcal{O}$ is the set of nodes and $\mathcal{E}$ is the set of edges. A node $O_i \in \mathcal{O}$ in the sequencing graph represents an operation, whose type τ_i and duration u_i are specified by the user. The type τ_i of the operation, e.g., mix, heat and filter, is predefined by the application. To execute an operation, the corresponding device must be built in the chip and the operation must be assigned to this device. An edge $e_{ij} \in \mathcal{E}$ from O_i to O_j in the sequencing graph specifies that O_i must be executed before O_j and the result of O_i is the input of O_j. If O_i and O_j are executed by different devices, the required fluid transportation must be performed by the channel network between devices.

Biochips have a huge advantage over the traditional manual experiment flow, where operations performed by humans are error-prone and inaccurate. Any inadvertent mistake in this manual process might ruin a complex experiment that may last for several days. In a biochip, the volumes of fluid samples and reagents are controlled accurately and fluid samples are moved to target devices reliably, all of which are managed by a microcontroller exactly following a given protocol. In addition, the miniaturized size of biochips makes them extremely portable, so that a complex lab can be integrated into a single chip and carried conveniently to perform on-the-spot tests to counter acute disease outbreaks such as the devastating Ebola virus disease a few years ago. Furthermore, reactions with fluid samples and reagents of tiny volumes take less time to complete than those with large volumes in tubes and in the traditional experiment flow, so that biochips are also more responsive in dealing with urgent situations. Moreover, biochips save precious reagents by performing operations at nanoliter level. For example, RNase inhibitor, a polyclonal antibody commonly used in reverse transcription polymerase chain reaction, cost 600 euros per milliliter in December 2014 (**?**).

The miniaturization of microfluidic biochips also has the potential of large-scale system integration. Already in 2008, a biochip array with 25K valves was accomplished (**?**), and recent advances in manufacturing technologies have led to a valve density of 1 million per cm^2 (**?**). A system integration of this scale enables long-aspired exhaustive diagnoses in identifying the illness of a patient by testing pathological samples with thousands of reagents simultaneously. This breakthrough will not only reduce the inaccuracy in medical diagnoses, where individual expertise and experience of doctors play an important role, but also change the current guess-then-test model of medical

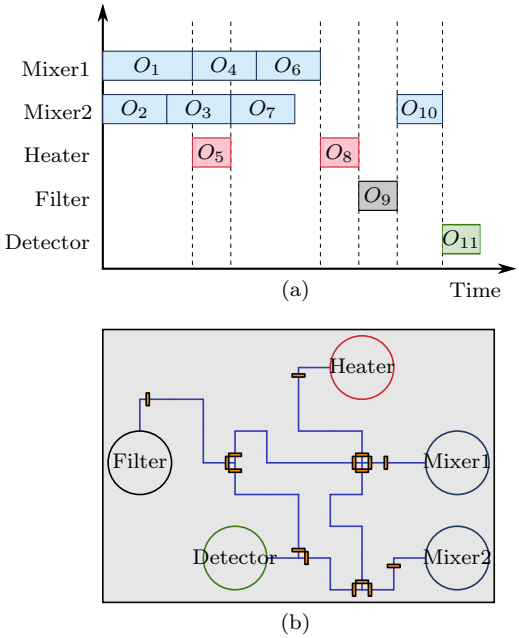

Figure 1.3: Synthesis of microfluidic biochips. (a) Scheduling. (b) Physical design.

treatment. In addition, such exhaustive diagnoses can be performed in small health-care centers routinely, due to the tremendously miniaturized chip size and lowered cost. With this exhaustive diagnosis model, illnesses can be detected at a very early stage and treatment cost can be reduced significantly as well.

Owing to their efficiency and cost-effectiveness, microfluidic biochips are reshaping many fields such as pharmacy, biotechnology and health care. In recent years, genomic bioassay protocols, such as nucleic-acid isolation, DNA purification and DNA sequencing, have been successfully demonstrated with microfluidic biochips. In addition, this technology has attracted a lot of commercial attention, e.g., from Illumina (**?**), a market leader in DNA sequencing. Accordingly, the International Technology Roadmap for Semiconductors (ITRS) 2015 (**?**) has recognized the importance of microfluidic devices as having a rapid growth in the next several years.

Synthesis of microfluidic biochips using computer algorithms

In a biochip, operations are executed by a given number of devices with time multiplexing, described as a schedule. For example, an execution of the bioassay illustrated in Figure 1.2 is shown in Figure 1.3(a), where two mixers, one heater, one filter, and one detector are available. According to the schedule,

the layout of a biochip, including the locations of devices and the transportation channels between them, can be determined to generate a physical design, as shown in Figure 1.3(b), where the devices are connected by a channel network controlled by valves.

The synthesis process above demonstrates that the schedule of operations of a bioassay determines the overall execution time. In addition, the fluid transportation between devices affects the structure of the channel network. Consequently, a holistic design automation flow is required to bridge the low-level components introduced by the microfluidics community with high-level real-world applications. In each step of this design automation flow, various design objectives should be optimized to achieve an efficient architecture for the biochip.

The synthesis flow of biochips is similar to the synthesis flow for integrated circuits (**?**). Therefore, researchers in the electronic design automation community have started to expand into this area in recent years (**? ?**). However, these research efforts are still in an early stage and many unique characteristics of microfluidic biochips have still not been considered.

Flow-based microfluidic architectures: the electronic view

In the microfluidics community, researchers are focusing on developing new technologies and new structures to build fundamental components and devices, such as valves and pumps (**? ?**). Prototype microfluidic biochips are also built very often to demonstrate the function and performance of new components and new devices. Another major focus of the microfluidics community is to increase the integration density of basic components. With the advance in MEMS technology, a large number of components such as valves can now be built in a single biochip (**?**). Unfortunately, the abundant available resources have mostly been left unexplored, because end users cannot use them without a system layer that presents an interface for user applications, similar to the scenario that an operating system is missing for computer users. On the other hand, the effort of the microfluidics research community has been spread out in exploring even more technologies for microfluidic biochips, leading to a flourishing but fragmented panorama in the research on microfluidics.

The status of the microfluidics community is similar to the early period of the semiconductor industry. At that time, researchers were exploring different materials

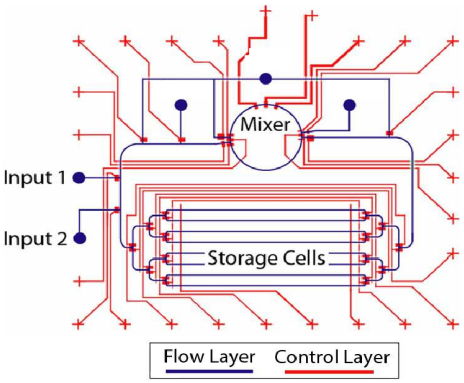

Figure 1.4: Computing-based biochip architecture containing a mixer and a dedicated storage unit with eight cells (**?**).

and device structures to build smaller but faster transistors. Thereafter, CMOS-based technology became dominant in this industry, while other technologies are employed only for specific applications. CMOS technology obtained its dominance because of its performance. However, the development of Electronic Design Automation (EDA) has supported the large-scale integration in design and manufacturing and made the computing resources available to designers successfully.

Observing the state of the art of microfluidic biochips, researchers from computer science and electrical engineering have started to bring their own computing models into microfluidic biochips. For example, the architecture of a microfluidic biochip from (**?**) is shown in Figure 1.4. In this architecture, the mixer functions as the computing unit and intermediate results from the mixer are stored in the dedicated storage unit. The cells in the storage unit are built from normal channels. At the ports of this storage unit, valves form multiplexers to direct fluid samples to enter into or leave from specific cells. This architecture emulates the classical von Neumann computer

architecture to build a biochemical computing system from basic components. However, this simple emulation forsakes many unique characteristics of flow-based biochips, leading to inefficient execution of bioassays.

Similar to the semiconductor industry, design automation tool chains are also needed to support the development of microfluidic biochips. In recent years, the electronic design automation community has tried to migrate the existing design methodologies for integrated circuits to microfluidic biochip design, covering the phases from high-level synthesis down to physical design. Although this top-down flow has served the

semiconductor industry in the past 50 years very successfully, fundamental changes should still be made to deal with specific requirements of biochips and take advantage of their unique features.

Flow-based microfluidic biochips: the unique characteristics

In microfluidic biochips, the inputs to an operation are fluid samples. Unlike electrical signals in integrated circuits, these fluid samples have a physical mass. In executing operations of a bioassay, fluid samples are processed with various operations, such as mixing, heating and detecting in different devices. The results of these operations are often fluid samples of different properties, so that inadvertent contamination between them should be avoided. The intermediate results of these operations should be stored in the chip temporarily in case they are not used immediately. Consequently, the physical mass and the variety of fluid samples become the major differences between biochips and integrated circuits, leading to several unique characteristics in biochip design.

Volume Management: In executing a bioassay, the volumes of fluid samples should be managed. Assume all the devices executing the bioassay in Figure 1.2 have a capacity ν. Each of the resulting samples of O_1 and O_2 thus has a volume ν. When these two fluid samples reach the device executing O_7, half of their volumes should be disposed of because the device only accepts a volume ν. This volume management is not stated explicitly in the sequencing graph, but must be dealt with implicitly according to the volumes of intermediate fluid samples and the capacities of devices.

Storage management: In the schedule in Figure 1.3(a), O_2 completes before O_5 does. The intermediate result of O_2 should be moved out of Mixer2 and stored somewhere temporarily so that the mixer can execute O_3. In the biochip shown in Figure 1.4, this storage function is fulfilled by moving the result of O_2 to the dedicated storage unit through a channel. In synthesizing biochips, if operations are not scheduled properly, many storage requirements may appear, leading to many transportation channels and a large storage unit. In contrast to a dedicated storage unit as shown in Figure 1.4, the storage function can actually be implemented using distributed transportation channels. In fact, a fluid sample can stay anywhere in a channel in the biochip until it is used by the next operation. This is a significant difference between biochips and electronic systems, where intermediate data can only be stored in special memory units, either flip-flops or RAM components. This observation can be confirmed by the storage cells

in the dedicated storage unit in Figure 1.4. These cells are built of normal channels but with valves at each end of a channel to control the store/fetch operations. Instead of forming a monolithic storage unit, these channels and valves can actually be distributed in the chip so that they can be used for storage when required, and for transportation otherwise, leading to better flexibility and wearing balancing.

Washing: Unlike electrical signals, fluid samples leave residue in channels after they travel through them. Before such a channel is reused by another fluid sample, it should be washed by neutral fluids such as silicon oil. Washing contaminated channels can be very flexible because several channel segments can be washed simultaneously if they form a connected graph while being isolated from the rest of the biochip that is executing other operations.

Flow-layer and control-layer codesign: In a flow-based biochip, valves are controlled by air pressure through control channels, e.g., the red channels in Figure 1.4. If all the valves are controlled independently, the routing of control channels in a complex design becomes very complicated. To solve this problem, control channels of some valves can be shared if operations can still be executed correctly. This sharing requires a codesign of the flow layer and the control layer to match the actuation patterns of valves.

State-of-the-art research on design automation for flow-based biochips using computer algorithms

Several methods have been proposed to synthesize flow-based biochips. The method in (**?**) proposes a top-down flow to generate a biochip architecture while minimizing the execution time of a bioassay. The flow channel routing problem considering obstacles is solved with an algorithm based on rectilinear Steiner minimum tree in (**?**). These methods still assume that intermediate fluid results can be stored automatically in a dedicated storage unit as in the biochip inspired by electronic design shown in Figure 1.4. The real storage process and its efficiency, however, have not been investigated.

To avoid contamination, a method based on path searching is introduced in (**?**) to wash devices and channel segments. This method still traces path sets and block-based partial washing has not been explored. The latter requires a co-optimization between operation scheduling and washing activities.

Control logic synthesis is investigated in (**?**) to reduce the number of control pins. The method in (**?**) minimizes pressure-propagation delay in the control layer

to reduce the response time of valves and synchronize their actuations. Furthermore, flow layer and control layer codesign is investigated in (**?**) to achieve valid routing results on both layers iteratively, and length-matching in routing control channels is considered in (**?**) as well. Since these methods do not consider operation scheduling, the number of control channels may still be large and consequently they might not be routed successfully.

Though the volume management problem in biochips has been explored as early as in 2008 (**?**), and later in (**?**) for the specific bioassay sample preparation, the optimization of volume management for general bioassays and the interaction of this task with fluid transportation for normal operations have not been taken into account.

When the unique characteristics of biochips are considered, the tasks of synthesizing flow-based biochips are entangled with each other. Consequently, a systematic design flow covering architectural synthesis, control layer design, washing and volume management should be explored, which is the major objective of this project.

Preliminary work

Observing the great potential of microfluidic biochips and the design automation challenges at the eve of their large-scale integration, I have initiated the research on biochips in the Institute for Electronic Design Automation at TUM. Applying the knowledge on design automation methods for IC design to microfluidic biochips, several preliminary ideas have been verified in our research group to synthesize efficient biochip architectures.

In the research community, we have pioneered the idea of distributed channel storage in flow-based biochips (**?**). We have also proposed to improve the reliability of biochips with a large-scale integration (**?**), where a fully reconfigurable valve array is used to execute operations and fulfill the functions of transportation and storage. Furthermore, we have introduced a path-based vector generation method for test of microfluidic biochips (**?**). Fault localization and design-for-testibility of microfluidic biochips have been addressed in (**?** **?**). Moreover, optimization of control logic to improve its efficiency and the overall portability of the microfluidic platform has been explored in (**?**).

To bridge microfluidic biochips with their applications, techniques are required to map bioassays to specific architectures. More importantly, the structures of bioassays

may influence biochip architectures because different sequencing graphs lead to different execution, transportation and storage requirements. Therefore, efficient algorithms are also needed to optimize biochip architecture and assay execution. In the past, our research group and the EDA institute had broad research activities in design automation for integrated circuits with well-recognized results. The developed algorithms and tools may also potentially benefit the research on microfluidic biochips, e.g., those for physical design (**?**), circuit test and tuning (**?**) (Nomination for Best Paper Award at DAC 2016), reliability (**?**), as well as hierarchical modeling and analysis (**?**).

Insulin stimulates the following processes:

- muscle and fat cells remove glucose from the blood,
- cells breakdown glucose via glycolysis and the citrate cycle, storing its energy in the form of ATP,
- liver and muscle store glucose as glycogen as a short-term energy reserve,
- adipose tissue stores glucose as fat for long-term energy reserve, and
- cells use glucose for protein synthesis.

Gene	GeneID	Length
human latexin	1234	14.9 kbps
mouse latexin	2345	10.1 kbps
rat latexin	3456	9.6 kbps

Table 1.1: title of table - Overview of latexin genes.

References