Design-for-Testability for Digital Microfluidic Biochips*

Tao Xu and Krishnendu Chakrabarty
Department of Electrical and Computer Engineering
Duke University, Durham, NC 27708, USA

***Abstract*-Testing is essential for digital microfluidic biochips that are used for safety-critical applications such as point-of-care health assessment, air-quality monitoring, and food-safety testing. However, the effectiveness of recently proposed test techniques for biochips is limited by the fact that current design methods do not consider testability. We introduce the concept of design-for-testability (DFT) for microfluidic biochips and propose a DFT method that incorporates a test plan into the fluidic operations of a target bioassay protocol. By using the testability-aware bioassay protocol as an input to the biochip design tool, the proposed DFT method ensures a high level of testability, defined as the percentage of the electrodes or functional units on the synthesized chip that can be effectively tested. We evaluate the DFT method using a representative multiplexed bioassay and the polymerase chain reaction.**

Keywords - biochip testing, DFT, functional testability, lab-on-chip, pin-constrained biochips

I. INTRODUCTION

The emergence of microfluidic biochips has led to the automation of laboratory procedures in biochemistry and the miniaturization of laboratory instruments [1,2]. Compared to traditional bench-top procedures, microfluidic biochips offer the advantages of low sample and reagent consumption, less likelihood of error due to minimal human intervention, high throughput, and high sensitivity. These devices are now being advocated for a wide range of applications such as high-throughput DNA sequencing, immunoassays and clinical chemistry, environmental toxicity monitoring and the detection of airborne contaminants, detection of explosives such as TNT, and point-of-care diagnosis [3].

Many commercially-available biochips today rely on continuous fluid flow in etched microchannels [2]. Some recent continuous-flow biochip products include the Topaz™ system for protein crystallization from Fluidigm Corporation, the LabChip system from Caliper Life Sciences, and the LabCD™ system from Tecan Systems, Inc. An alternative category of microfluidic biochips relies on "digital microfluidics", which is based on the principle of electrowetting-on-dielectric [1]. By manipulating discrete droplets of nanoliter volume using a patterned array of electrodes, miniaturized bioassay protocols (in terms of liquid volumes and assay times) are mapped and executed on a microfluidic chip. Digital microfluidic biochips are reconfigurable and multifunctional, and they offer continuous sampling and analysis capabilities for on-line and real-time chemical/biological sensing.

Recent years have seen a steady increase in the system complexity of digital microfluidic biochips [4-7]. A prototype has been developed for protein crystallization, which requires the concurrent execution of hundreds of operations [6,8]. A commercially available droplet-based biochip (using dielectrophoresis) embeds more than 600,000 20 μm by 20 μm electrodes with integrated optical detectors [9].

Dependability is an important system attribute for biochips. It is especially needed for safety-critical applications such as point-of care diagnostics, health assessment and screening for infectious diseases, air-quality monitoring, and food-safety tests, as well as for pharmacological procedures for drug design and discovery that require high precision levels. Therefore, microfluidic biochips must be tested adequately after manufacture and during field operation.

A number of test methods for digital microfluidic biochips have been proposed [10-14]. However, the effectiveness of these test techniques is limited by the fact that current design methods do not consider testability. For example, most of these methods assume that the chip under test is a rectangular array controlled using a direct-addressing scheme, i.e., each electrode on the array is connected to an independent control pin. This method provides the maximum freedom for test-droplet manipulation, but it requires a large number of control pins. For example, a total of 10^4 pins are needed to independently control the electrodes in a 100×100 array.

To reduce production cost, unused electrodes are often removed from the rectangular array, resulting in an irregular chip layout. For example, in Fig. 1, several electrodes have been removed to reduce cost. To further reduce the number of control pins, pin-constrained design techniques are used in practice, whereby multiple electrodes are connected to a single control pin [16-19]. These design methods achieve a significant reduction in the number of input pins needed for controlling the electrodes. However, as a trade-off, droplet manipulation steps must satisfy additional constraints. These constraints can result in test procedures being either completely ineffective or effective only for a small part of the chip. As a result, chip testability is significantly reduced.

To tackle the above testability problem, we introduce the concept of design-for-testability (DFT) for biochips. The motivation of DFT for biochips is analogous to that for integrated circuits (ICs). In the early days of IC design, chip area and performance were the primary concerns for chip designers, and testing was only an afterthought. However, as chip complexity increased, test problems were greatly exacerbated and DFT became essential. Compared to the IC industry, digital microfluidics technology is still in its infancy. However, tremendous growth has been predicted for this technology and biochips for clinical diagnostics and cell sorting are now appearing in the marketplace [9, 15]. As these devices become more complex, the need for DFT will be increasingly felt.

In this paper, we provide a DFT solution to facilitate the testing of digital microfluidic biochips. We propose a test-aware design method that incorporates test procedures into the fluidic manipulation steps in the target bioassay protocol. By applying pin-constrained design to the testability-aware bioassay protocol, the proposed method ensures that the resulting chip layout supports the effective execution of test-related droplet operations for the entire chip. Therefore, the proposed DFT method allows design of pin-constrained biochips with a high level of testability with negligible overhead in terms of the number of control pins. The proposed design method also ensures that DFT does not add to the assay completion time for the target biochemical application.

The rest of the paper is organized as follows. Section II provides an overview of the digital microfluidic platform. Pin-constrained design techniques are discussed in Section III. In Section IV, we review prior work on biochip testing. Section V explains the testability problem. In Section VI, we introduce the concept of DFT for biochips and propose the testability-aware design method. In Section VII, we apply the proposed test-aware design method to a multiplexed bioassay and a PCR assay, and present simulation results. Finally, conclusions are drawn in Section VIII.

*This work was supported in part by the National Science Foundation under grant no. CCF-0541055.

1093-0167/09 $25.00 © 2009 IEEE
DOI 10.1109/VTS.2009.16

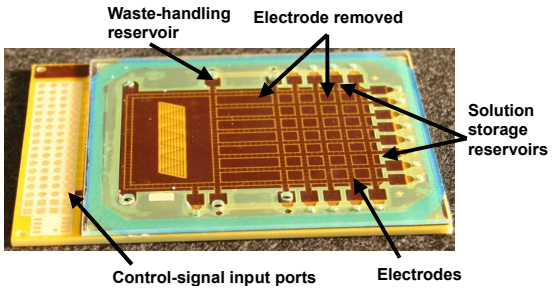

Fig. 1: A fabricated digital microfluidic biochip [15].

II. DIGITAL MICROFLUIDIC BIOCHIPS

A digital microfluidic biochip utilizes the phenomenon of electrowetting to manipulate and move nanoliter droplets containing biological samples 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. 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.

By varying the patterns of control voltage activation, many fluid-handling operations such as droplet merging, splitting, mixing, and dispensing can be easily executed. For example, mixing can be performed by routing two droplets to the same location and then turning them about some pivot points. 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 digital microfluidic arrays to monitor colorimetric bioassays [3].

III. PIN-CONSTRAINED DESIGN

As discussed in Section I, a direct-addressable chip requires a large number of independent control pins. Product cost, however, is a major market driver due to the one-time-use (disposable) nature of most biochips. Thus, the design of pin-constrained digital microfluidic arrays is important for the emerging marketplace.

Pin-constrained design of digital microfluidic biochips was first proposed and analyzed in [16]. The number of control pins for a fabricated electrowetting-based biochip is minimized by using a multi-phase bus for the fluidic pathways. Every nth electrode in an n-phase bus is electrically connected. Thus, only n control pins are needed for a transport bus, irrespective of the number of electrodes connected to it.

An alternative method based on a cross-reference driving scheme is presented in [17, 18]. The electrode rows are patterned on both the top and bottom plates, and placed orthogonally. In order to drive a droplet along the X-direction, electrode rows on the bottom plate serve as driving electrodes, while electrode rows on the top serve as reference ground electrodes. The roles are reversed for movement along the Y-direction. This method allows control of an $N \times M$ grid array with only $N+M$ control pins.

Another pin-constrained design method is based on the partitioning of the microfluidic array and the assignment of a small number of control pins to a large number of electrodes in each partition. The partitioning algorithm is based on the concept of "droplet trace", which is extracted from the scheduling and droplet routing results produced by a synthesis tool [19]. The key idea is to "virtually" partition the array into regions. At any given time, partitions use non-overlapping sets of pins.

More recently a broadcast-addressing-based design technique for pin-constrained multi-functional biochips has been proposed [20]. This method provides high throughput for bioassays and it reduces the number of control pins by identifying and connecting control pins with "compatible" actuation sequences.

IV. RELATED PRIOR WORK ON BIOCHIP TESTING

The testing of microfluidic biochips has recently been investigated. These test methods add fluid-handling aspects to MEMS testing techniques [10, 11]. Test methods have been proposed for both continuous-flow and digital microfluidic biochips. Fault models and a fault simulation method for continuous-flow microfluidic biochips have been proposed in [21]. For digital microfluidic chips, techniques for defect classification, test planning, and test resource optimization were first presented [22]. A testing method based on Euler paths in graphs is proposed in [23]. This method maps a digital microfluidic biochip to an undirected graph and a test droplet is routed along the Euler path derived from the graph to pass through all the cells in the array. Fault diagnosis is carried out using multiple test-application steps and adaptive Euler paths. The test methods discussed in [22,23] are referred to as structural test, since they route test droplets to all the electrodes in the array to ensure structural integrity.

More recently, several techniques have been presented for the functional testing of digital microfluidic biochips [14]. These techniques address fundamental biochip operations such as droplet dispensing, droplet transportation, mixing, splitting, and capacitive sensing. Functional testing is carried out using parallel droplet pathways, and it leads to qualified regions where synthesis tools can map microfluidic functional modules.

V. TESTABILITY OF A DIGITAL MICROFLUDIC BIOCHIP

The test methods discussed in Section IV are applicable only to direct-addressable chips. For pin-constrained chips, due to the constraints introduced by sharing of input pins by the electrodes, these test procedures can be either completely ineffective or effective for only a small part of the chip.

To evaluate the effectiveness of a test procedure, we define a parameter referred to as testability. Given a specific test method, the *testability* of a chip design is defined as the ratio of testable electrodes/functional units to the total number of electrodes/functional units on the chip, where a functional unit is defined as a cluster of adjacent electrodes that can carry out a specific type of fluidic operation. Depending on the test method and chip functionality, chip testability can be classified into two categories, namely structural testability and functional testability.

Structural testability is defined as the percentage of testable electrodes on the chip during a structural test. An electrode is considered "testable" if it can be traversed by the test droplet. Note that for most biochips, including pin-constrained chips, any on-chip electrode has to be traversed by at least one droplet in order to carry out the fluidic operations mapped on it. This means it can also be traversed by the test droplet. Therefore, most chip designs can achieve a structural testability of 100%.

Functional testability is defined as the percentage of testable functional units on a chip in a functional test procedure. High testability indicates that the test method can probe the functionality of the chip thoroughly and identify a large number of qualified regions for a target application, which in turn contributes to increased flexibility for design and fault tolerance. A functional

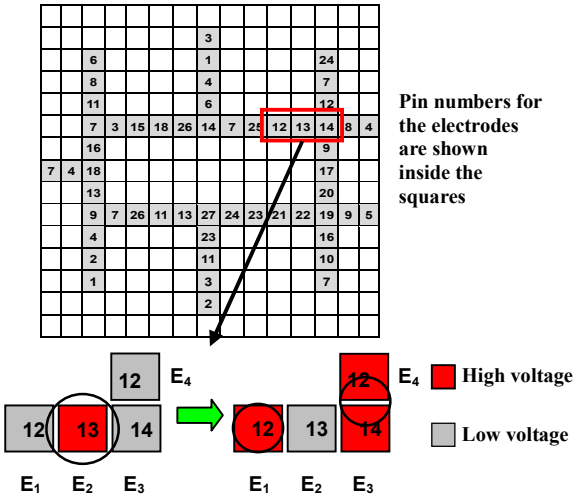

Fig. 2: An example of an untestable functional unit on a pin-constrained chip for multiplexed assay.

unit is considered to be "testable" if the test-related droplets can be manipulated to carry out the target fluidic operations on it. These fluidic operations in the functional mode are always possible on a direct-addressable chip. However, for a pin-constrained chip, due to constraints introduced by the sharing of input control pins by electrodes, carrying out these functional-test operations on some functional units can result in unintentional droplet manipulations. We use an example to explain this problem.

Fig. 2 shows a pin-constrained chip design for a representative protein-dilution assay [20]. The functional test procedure requires a splitting operation to be executed on the highlighted functional unit. To do this, we first activate Pin 13 to hold a test droplet at E_2. Next, we deactivate Pin 13 and activate Pin 12 and Pin 14 to split the test droplet into two small droplets seated on E_1 and E_3. However, E_4 is also charged by activating Pin 12. As a result, the split droplet that is supposed to be seated on E_2 will be moved unintentionally to the boundary of E_4 and E_3. This type of problem is referred to as *electrode interference*.

Due to the above electrode interference problem, functional test cannot be applied to all the functional units in a pin-constrained chip design. Therefore, functional testability for a pin-constrained chip is usually less than 100%. Note that the reduction in testability is due to the conflicts between the fluidic operation steps required by functional test and the constraints on droplet manipulations introduced by the mapping of pins to electrodes. Different mappings for a pin-constrained chip lead to different untestable functional units, thereby different levels of chip testability. For example, the untestable functional unit shown in Fig. 2 can be made testable by connecting electrode E_4 to a different control pin, e.g. Pin 15. Therefore, we can conclude that the key to increasing functional testability is to generate a test-friendly pin assignment that results in a small number of untestable functional units. To do this, test procedures must be considered early during chip design.

VI. TESTABILITY-AWARE PIN-CONSTRAINED CHIP DESIGN

In this section, we propose a DFT solution to the functional testability problem described in Section V.

A. Design Method

Our key idea is to incorporate fluidic operations required by functional test into the fluidic manipulation steps for the bioassay. Since these test-aware fluidic manipulation steps are provided as

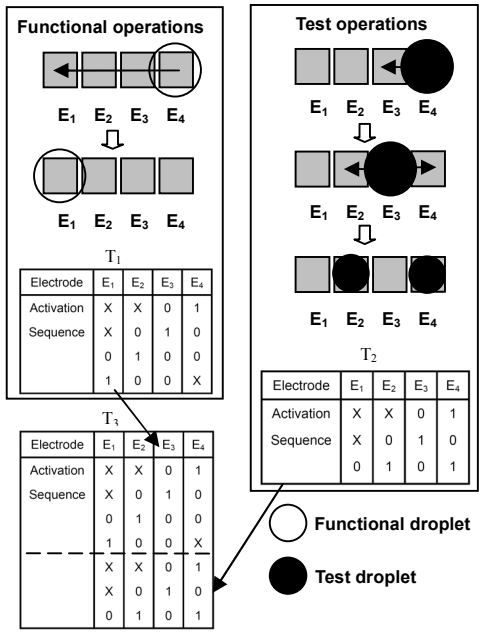

Fig. 3: Illustration of the influence by adding test operations to the bioassay.

input to a pin-assignment design method, the resulting test-aware pin-constrained chip design guarantees a test-friendly pin assignment that supports all the fluidic operations required for functional test, thereby ensuring full testability.

The pin assignment in the proposed test-aware design method is based on the broadcast-addressing pin-constrained chip design technique presented in [20]. In this design method, fluidic manipulation steps in a target bioassay, represented by the droplet schedule and droplet-routing steps, are stored in the electronic controller in the form of electrode-activation sequences. Each bit of the sequence represents the status of an electrode at a specific time-step. The status can be either "1" (activate), "0" (deactivate) or "X" (don't care), which can be mapped to either "1" or "0". For each electrode on the chip, its activation sequence can be represented using the above three values. Each sequence can contain several don't-care terms, which can be replaced by "1" or "0". By careful replacing these don't-care terms, multiple activation sequences can be made identical. Therefore, the corresponding electrodes can be connected to a single control pin. The broadcast-addressing method achieves a significant reduction in the number of control pins, and the resulting pin assignment ensures the correct execution of all fluidic operations in the bioassay.

We first consider the functional test procedure as a separate bioassay. The fluidic operations required by the test procedure are derived from the scheduling and routing steps related to the test droplets. Next, we merge these fluidic operations with the droplet manipulation steps needed for the target bioassay. The merging can be carried out by attaching the electrode-activation sequences for the test procedure to the electrode-activation sequences for the target bioassays. For each electrode in the array, its activation sequence during the test procedure is added to that for the target bioassay to form a longer sequence. If these longer electrode-activation sequences are provided as input to the broadcast-addressing method, the resulted chip design will support not only the target bioassay but also the test operations.

We use an example to illustrate the details of the above DFT method. Fig. 3 shows a linear array consisting of four electrodes. A

simple "routing assay" is mapped to the array, where a droplet is to be routed from E_4 to E_1, one electrode per step. We first list the activation sequence for each electrode (Table T_1) in Fig. 3. Next we add a splitting test on E_3. The electrode-activation sequences for the splitting test are shown in Table T_2 of Fig. 3. These activation sequences are then combined with the activation sequences in T_1. The resulted longer activation sequences are listed in table T_3. The broadcast-addressing method is then applied to replace the don't-care terms in T_3 and generate the eventual pin assignment.

Note that the addition of test operations into the bioassay may result in an increase number of control pins, compared to a test-unaware design using broadcast addressing. As shown in Fig. 3 (Table T_1), before the splitting test is added, we can map the two don't-cares in the activation sequence for E_1 with "10" and map the don't-care in the activation sequence for E_4 with "1" to make the two sequences identical. Therefore, the corresponding electrodes E_1 and E_4 can be connected to a single control pin. As a result, only three control pins are needed to control the linear array. However, when the splitting test is added, activation sequences in Table T_3 become incompatible. Therefore, they have to be controlled independently. The linear array now requires four control pins.

B. Euler-Path-Based Functional Test Method for Irregular Chip Layout

The test operations used in the above testability-aware pin-constrained design method can be determined using the functional test method in [14]. However, this approach requires a rectangular array structure for the chip under test. As discussed in Section I, to reduce cost in practical designs, unused electrodes are often removed from the array, resulting in an irregular chip layout. Irregular layouts also result from the need for allocating routing tracks under the fluidic layer for connecting the electrodes to chip pins. In this subsection, we propose an Euler-path-based method for the functional testing of such irregular chip layouts.

For simplicity, we focus on the functional testing of two widely used microfluidic modules—mixers and splitters. According to [14], a mixing functional test can be reduced to a droplet-merging test, which actuates a series of three adjacent electrodes to determine whether two droplets can be merged on them. A split operation can be viewed as the reverse of droplet merging. Consequently, these two tests can be combined into a unified splitting-and-merging test application procedure.

In a splitting-and-merging test for a single functional unit, a test droplet is routed to the center electrode of the three-electrode cluster, split, merged, and finally routed back to a detection site for test readout. To carry out mixing and splitting functional test for a chip, this basic splitting-and-merging test needs to be a carried out on every three-electrode cluster on the chip.

For a rectangular array, multiple splitting-and-merging tests can be carried out in parallel on a row/column of electrodes, as shown in Fig. 5. However, parallel testing is not always feasible on an irregular-shape chip layout. Instead, the splitting-and-merging steps have to be carried out one at a time. All the functional units need to be targeted for full testability. However, overtesting must be avoided, i.e., a functional unit should not be tested repeatedly. To meet these criteria, we carry out the splitting-and-merging test along the Euler path of the chip layout.

Given an irregular chip layout, the Euler-path-based functional test method first maps it to an undirected graph and extracts the Euler path [23]. An Euler path traverses every edge in the graph exactly once. Next the mixing-and-splitting test is applied to the functional units along the Euler path, one at a time, until all the functional units are covered, as shown in Fig. 6(a). Note that by following the above steps, the test droplet will traverse all the electrodes on the chip. Therefore, structural test is also accomplished.

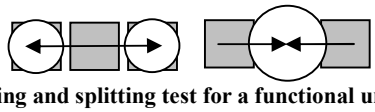

Fig. 4: Mixing and splitting test for a functional unit.

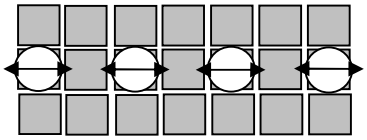

Fig. 5: Parallel mixing and splitting test for a row of electrodes.

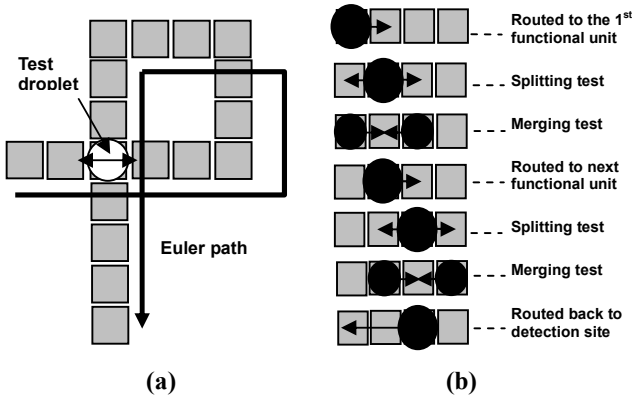

Fig. 6: (a) Mixing-and-splitting test of functional units along the Euler path of a chip (b) Testing functional units in groups of two.

To reduce the test-completion time, the functional units can be tested in groups. After the splitting-and-mixing test for a target functional unit is completed, the test droplet can be used to test the adjacent functional units instead of being routed to the source reservoir. Therefore, the test droplet is routed back to the source reservoir for test readout (Fig. 6(b)) only after a group of functional units is tested.

The test efficiency depends on the size of a group of functional units being targeted by the same test droplet. A large group can be targeted to reduce test time. As a trade off, this group-testing scheme leads to reduced resolution for diagnosis. We can appropriately select the size of these groups to meet different test requirements.

VII. SIMULATION RESULTS

In this section, we evaluate the proposed Euler-path-based functional testing method and the testability-aware design method by applying them to two target applications: a multiplexed immunoassay and the polymerase chain reaction (PCR) procedure.

Each assay is first mapped to a 15×15 electrode array controlled using the direct-addressing scheme. Unused electrodes are removed from the array, resulting in irregular chip layouts. Next, the proposed Euler-path-based functional test method is applied to obtain a test plan for the chip. Finally, the test-aware design method is used to generate a pin-constrained design with a high level of testability.

A. Multiplexed Assay

We first map a recently demonstrated multiplexed biochemical assay used for in-vitro measurement and other antigens in human physiological fluids, which is of great importance for clinical diagnosis. For instance, a change in regular metabolic parameters

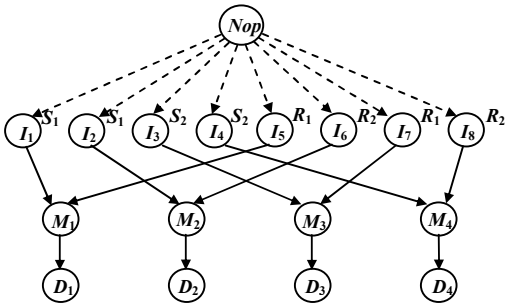

Fig. 7: Sequencing graph model for a multiplexed bioassay. S_1, S_2 are samples, R_1, R_2 are reagents, M_1~M_4 are mixing operations, and D_1 ~D_4 are detection operations.

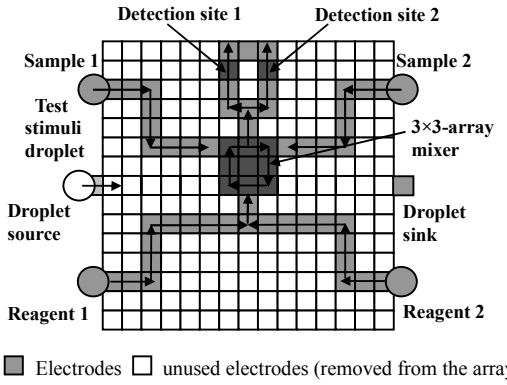

Fig. 8: Mapping of a multiplexed bioassay to a 15×15 array.

in a patient's blood can signal organ damage or dysfunction prior to observable microscopic cellular damages or other symptoms. A portable, inexpensive biochip can be used for carrying out multiplexed bioassays for rapid and point-of-care diagnosis of such disorders. The multiplexed assay consists of a glucose assay and a lactate assay based on colorimetric enzymatic reactions. Fig. 7 shows the flowchart for the multiplexed assays in the form of a sequencing graph. For each sample or reagent, two droplets are dispensed into the array. Four pairs of droplets, i.e., $\{S_1, R_1\}$, $\{S_1, R_2\}$, $\{S_2, R_1\}$, $\{S_2, R_2\}$, are routed together in sequence for the mixing operation. Mixed droplets are finally routed to the detection site for analysis. A depiction of the droplet pathways for multiplexed glucose and lactase assays is given in Fig. 8.

In the multiplexed assay, eight droplets (two droplets from each sample/reagent) are dispensed and routed to the mixer located at the center. Next, four mixing and detection operations are carried out in a pipeline manner. We assume that the droplets are transported at the rate of 1 electrode/second, i.e., 1 Hz.

Next we apply the proposed Euler-path-based functional test method to the above chip layout. To investigate the influence of the number of electrodes in each test group on the test frequency, five iterations of Euler-path-based functional test are carried out. Electrodes are tested in groups of 1-5, respectively. The test completion times (assuming test-droplet routing frequency of 10 Hz) are shown in Fig. 9.

Fig. 9 shows that a significant reduction in test completion time is achieved by increasing the number of electrodes in each test group. For example, by testing the electrodes in groups of five instead of testing one by one, the test completion time drops sharply from 332.8 seconds to 98.2 seconds, i.e., a 71% reduction. Note that as a trade off, whenever an error is observed, we can only determine a group of five candidate defective functional units.

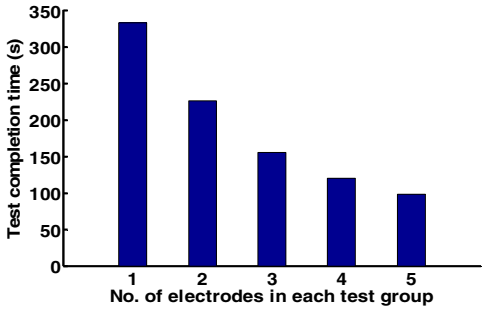

Fig. 9: Comparison of test completion time for the Euler-path-based functional test method.

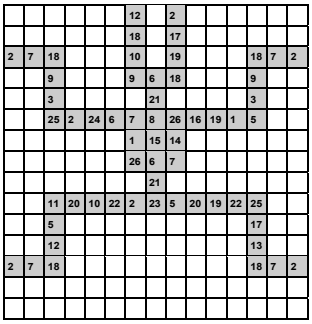

Fig. 10: Pin assignment for the multiplexed assay chip obtained using the testability-aware design method.

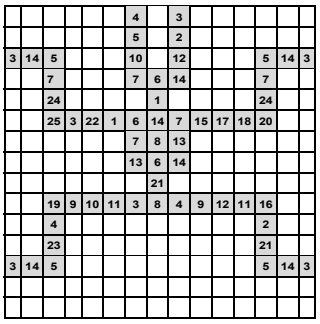

Fig. 11: Pin assignment for the multiplexed assay chip obtained using test-oblivious broadcast-addressing method.

Nevertheless, even such coarse-grained diagnostic information is useful in practice for dynamic reconfiguration.

Next we apply the test-aware design method to generate a pin assignment for the chip layout in Fig. 8. The test-droplet routing sequences derived from the Euler-path-based functional test are combined with the bioassay schedule. The pin assignment results are generated as shown in Fig. 10. For comparison, the pin assignment generated using the test-oblivious broadcast-addressing method of [20] is shown in Fig. 11.

As shown in Fig. 10, the pin assignment resulting from the test-aware design method uses 26 control pins, i.e., only one more control pin than test-oblivious pin assignment (Fig. 11). The test-aware design achieves 100% functional testability while the test-oblivious result achieves functional testability of only 76%.

B. Polymerase Chain Reaction (PCR)

For the second assay, we use the mixing stages of the PCR. These stages are used for rapid enzymatic amplification of specific DNA strands. Recently, the feasibility of performing droplet-based PCR on digital microfluidics-based biochips has been successfully demonstrated [15]. Its assay protocol can be modeled by a

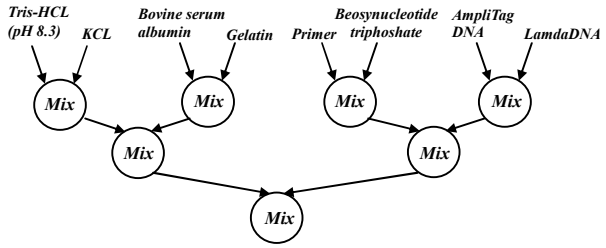

Fig. 12: Sequencing graph for the mixing stage of PCR.

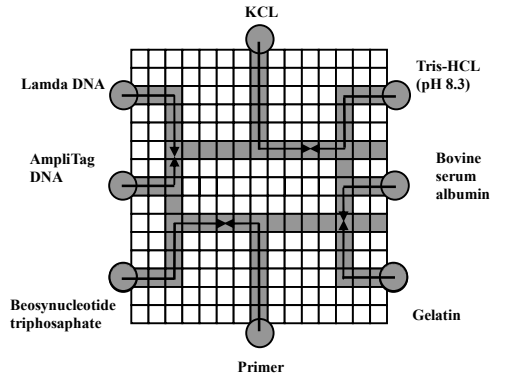

Fig. 13: Mapping of the PCR assay on a 15×15 array.

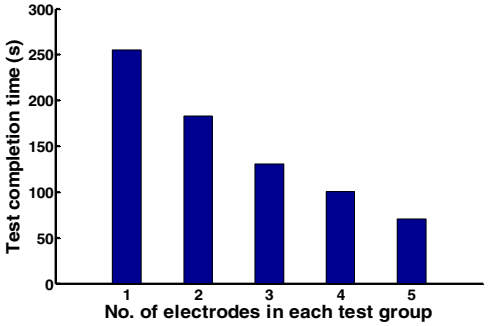

Fig. 14: Comparison of test completion time for the Euler-path-based functional test method.

sequencing graph, as shown in Fig. 12. Mapping the protocol on to the array, we obtain the chip layout shown in Fig. 13.

Next we apply the proposed Euler-path-based functional test method to the above chip layout. Again, five iterations of Euler-path-based functional test are carried out. The test completion times are shown in Fig. 14. As in Fig. 9, a significant reduction of test-completion time is achieved as the number of electrodes in each test group increases.

Next we apply the test-aware and test-unaware pin-constrained design methods to the chip layout of Fig. 13. The same number of control pins (15) is needed for both methods, indicating zero pin-count overhead for DFT. However, test-oblivious pin assignment allows provides functional testability of only 84%, while the test-aware methods achieves 100% testability. Therefore, by using DFT, we can qualify a larger chip area for use by synthesis tools with negligible penalty in the number of pins. The proposed design method also ensures that DFT does not add to the assay completion time for the target biochemical application.

VIII. CONCLUSIONS

We have introduced the concept of design-for-testability for microfluidic biochips. We have presented a DFT method method that allows the design of a pin-constrained biochip with full functional testability. An Euler-path-based functional test method, which allows functional testing for irregular chip layouts, has also been presented. We have evaluated the DFT and functional methods for a multiplexed bioassay and the PCR procedure. We have demonstrated that the DFT method introduces negligible overhead in terms of the number of control pins and it does not add to the assay completion time for the target biochemical application.

REFERENCES

[1] R. B. Fair et al, "Electrowetting-based on-chip sample processing for integrated microfluidics," *Proc. IEDM*, pp. 32.5.1-32.5.4, 2003.
[2] J. Zeng and T. Korsmeyer "Principles of droplet electrohydrodynamics for lab-on-a-chip", *Lab on a Chip*, vol. 4, issue, 44, pp. 265-277, 2004
[3] R. B. Fair et al., "Chemical and biological applications of digital-microfluidic devices". *IEEE Design & Test of Computers*, vol. 24, issue 1, pp. 10-24, 2007.
[4] J. Berthier, *Microdrops and Digital Microfluidics*, William Andrew Publishing, Norwich, NY, 2008.
[5] J. Gong et al., "Portable digital microfluidics platform with active but disposable lab-on-chip," *Proc. IEEE MEMS*, pp. 355-358, 2004.
[6] T. Xu et al, "Digital microfluidic biochip for protein crystallization", *IEEE-NIH Life Science Systems and Applications Workshop*, MD, 2007.
[7] I. B.-Nad et al., "Digital microfluidics for cell-based assays", *Lab on a Chip*, vol. 8, pp. 519-526, 2008.
[8] T. Xu et al., "Design and optimization of a digital microfluidic biochip for protein crystallization", *Proc. IEEE/ACM International Conference on Computer-Aided Design*, pp. 297-301, 2008.
[9] Silicon Biosystems, http://www.siliconbiosystems.com.
[10] N. Deb and R. D. Blanton, "Analysis of failure sources in surface-micromachined MEMS", *Proc. International Test Conference*, pp. 739-749, 2007.
[11] A. Dhayni et al., "Pseudorandom functional BIST for linear and nonlinear MEMS", *Proc. Design Automation and Test in Europ*, pp. 664-669, 2006.
[12] S. Mir et al., "Extending fault-based testing to microelectromechanical Systems", *Journal of Electronic Testing: Theory and Applications*, vol. 16, pp. 279-288, 2000.
[13] T. Xu and K. Chakrabarty, "Parallel scan-like test and multiple-defect diagnosis for digital microfluidic biochips", *IEEE Transactions on Biomedical Circuits and Systems*, vol. 1, pp. 148-158, June 2007.
[14] T. Xu and K. Chakrabarty, "Functional testing of digital microfluidic biochips", *Proc. IEEE International Test Conference*, 2007.
[15] Advanced Liquid Logic, Inc, http://www.liquid-logic.com.
[16] V. Srinivasan et al., "An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids". *Lab on a Chip*, pp. 310-315, 2004
[17] S. K. Fan et al., "Manipulation of multiple droplets on N×M grid by cross-reference EWOD driving scheme and pressure-contact packaging", *Proc. IEEE MEMS Conf*., pp. 694-697, 2003.
[18] T. Xu and K. Chakrabarty, "A droplet-manipulation method for achieving high-throughput in cross-referencing-based digital microfluidic biochips", *IEEE TCAD*, vol. 27, no.11, pp. 1905-1917, November 2008.
[19] T. Xu et al., "Automated design of pin-constrained digital microfluidic biochips under droplet-interference constraints", *ACM Journal on Emerging Technologies in Computing Systems*, 2007, vol. 3, Article 14.
[20] T. Xu and K. Chakrabarty, "Broadcast electrode-addressing for pin-constrained multi-functional digital microfluidic biochips", *Proc. IEEE/ACM Design Automation Conference*, pp. 173-178, 2008.
[21] H. G. Kerkhoff and M. Acar, "Testable design and testing of micro-electro-fluidic arrays", Proc. IEEE VTS, 2003, pp. 403-409.
[22] F. Su et al., "Ensuring the operational health of droplet-based microelectrofluidic biosensor systems", *IEEE Sensors*, 2005, vol. 5, pp. 763-773.
[23] F. Su et al., "Defect-oriented testing and diagnosis of digital microfluidics-based biochips", *Proc. IEEE International Test Conference*, pp. 487-496, 2005.