Cyberphysical Adaptation in Digital-Microfluidic Biochips

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***Abstract*—Microfluidic biochips offer an unprecedented opportunity to handle biochemical fluids on-chip for real-time clinical diagnostics using techniques such as flash chemistry. The past decade has seen significant progress in disease assessment and the recognition of target molecules using such devices; however, until recently, defects, erroneous fluidic operations, and inherent uncertainties remained a major barrier to the adoption and deployment of these devices. This paper describes recent advances in cyberphysical adaptation and a vision for a multi-layered architecture for cyberphysical microfluidic biochips. A cyberphysical design and optimization technique for gene-expression analysis and epigenetics is presented. This paper shows how technology has advanced from manipulating droplets on a chip to carrying out realistic on-chip biochemistry.**

I. Introduction

Biomolecular quantitative analysis has been a fundamental toolbox for studying cancer transcriptomic alterations and pathogen biology. To make use of this approach, a large number of studies has been devoted to advance microfluidic technologies that offer the benefits of reduced reagent and sample usage, lower cost, and portability [1]. Digital-microfluidic biochips (DMFBs) are especially promising due to their manufacturability, programmability, and ease of cyberphysical integration [2]–[5]—such features are critical for today's complex microbiology research.The ultimate goal of such efforts towards miniaturization is to perform complete sets of biochemical assays on the device, leading to portable device platforms for point-of-care (POC) setting (e.g., POC diagnostics and POC pathogen detection) [6]; in other words, make autonomous microfluidic devices a reality.

However, the realization of an autonomous digital-microfluidic system for molecular quantitative analysis requires further advances in cyberphysical integration and real-time data analysis. Recent work on cyberphysical DMFBs is limited to either error recovery or termination control of biochemical assays such as polymerase chain reaction (PCR) [3], [7]–[9]. More specifically, recent experimental demonstrations of run-time adaptation have not considered control flow to incorporate the specific "if-then-else" requirements of multiple sample pathways in molecular biology applications [10], [11]. A recent study demonstrates the need to empower cyberphysical DMFBs with an interactive firmware layer that can collect data from on-chip sensors, perform real-time data analysis, and guide resource allocation with appropriate decisions [12]. The transition to such an autonomous platform can be facilitated through design support for intelligent real-time decision-making in a multi-assay setting; this breakthrough will ensure that a diverse collection of protocol paths can be traversed. Advances in such cyberphysical design techniques for these platforms will push the frontiers in several key application areas, including genome-wide screening, epigenetic inheritance and cancer research, and proteomic analysis.

Early proposed cyberphysical designs of DMFBs consist of two main functional components: (1) sensor-based feedback that ensures real-time error detection; (2) intelligent control software that constructs the cyber space. Unfortunately, this design approach does not incorporate higher-level decision-making at the protocol level that can take advantage of a layered cyberphysical system (CPS) perspective. As a consequence, while significant advances have been made on optimization algorithms and designs tools for DMFBs, there exists a considerable gap between the promise of biochip design automation—that is typically limited to managing "droplets on a chip"—and actual benefit available to users in application areas such as quantitative gene-expression analysis [12]. Motivated by this need, it is necessary to rethink cyberphysical system architecture in DMFBs so that more generic experimental objectives such as quantitative analysis are supported, and the required level of dynamic adaptation is fulfilled. Fig. 1 highlights the difference between traditional cyberphysical DMFBs [3] and our vision for real-time quantitative analysis.

II. Architectural Design of Cyberphysical DMFBs

Our premise is that significant rethinking in system design is needed to ensure CPS adaptation for quantitative analysis on-chip. Fig. 2 illustrates the proposed CPS-inspired 5-layer $\mathcal{C}^5$ (based on the C for each level) architecture. Today's design methods incorporate online biochemistry-on-chip synthesis, which provides reconfiguration capability to recover from operational errors (Level IV in Fig. 2). However, there is still a gap between the physical space and online synthesis, which impedes the use of DMFBs for quantitative analysis due to the lack of autonomous data analysis and intelligent decision-making. There is a need to explore algorithmic innovations that fill the gap between the control and monitoring of the physical space on one side, and the cyber space (i.e., online biochemistry-on-chip synthesis) on the other side. Coupling the firmware with online synthesis will potentially open new

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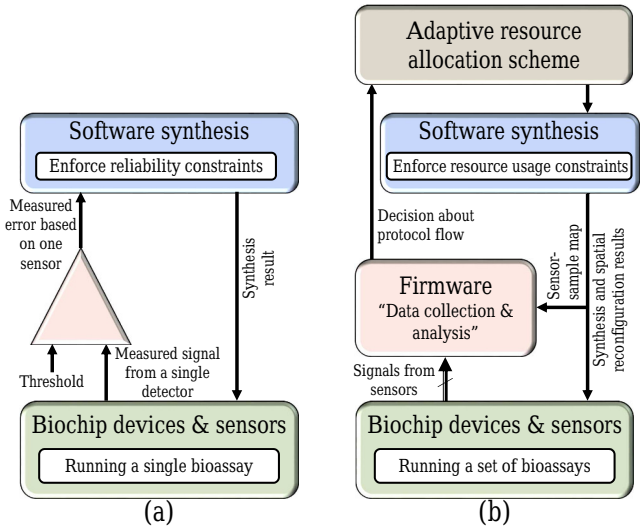

Fig. 1: A comparison between cyberphysical DMFBs targeted for: (a) traditional error recovery in early designs [3], [5]; and (b) real-time quantitative analysis [12].

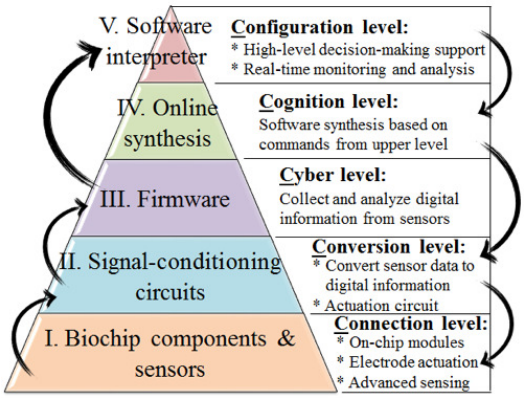

Fig. 2: The proposed unified 5-layer ($\mathcal{C}^5$) architecture for cyberphysical DMFBs.

opportunities for dynamic synthesis. For example, the need for short time-to-result might require the prioritizing and selection of samples for detection. These ideas can also be extended to prioritize the bioassays selected for synthesis in a multi-assay setting.

An important objective of such research efforts is to integrate these levels to enable the seamless on-chip execution of complex biochemical protocols. As shown in Fig. 2, each level is expected to play a distinct role. With the integration of sensors at the hardware level (Level I), there is a need to provide analog signal acquisition and digital signal processing capabilities to transform the received signals into readable data. This can be achieved via a signal conditioning level (Level II). Previous designs of cyberphysical DMFBs have attempted to integrate this level with the system infrastructure, but only for the limited purpose of error recovery.

The uppermost level (Level V) will be the system coordinator. It will be responsible for adapting the flow of protocol execution based on the decisions conveyed from the firmware (Level III). Adaptation is therefore supported by an application model, which keeps track of application progress,

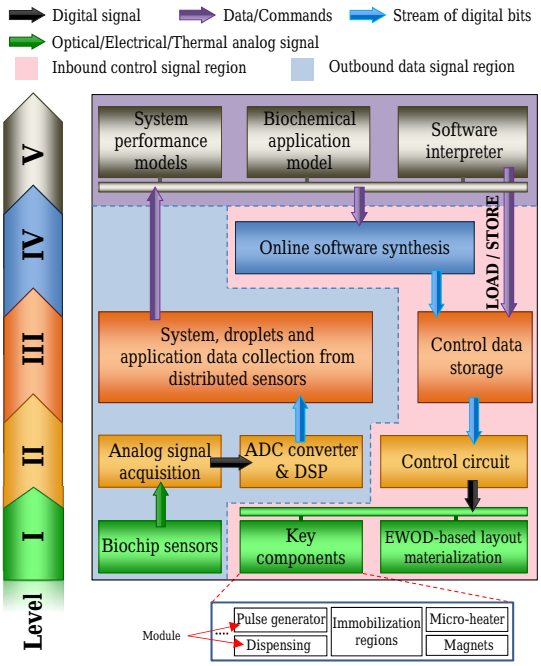

Fig. 3: The interactions among the various levels of the $\mathcal{C}^5$-compliant system infrastructure.

and a performance model that keeps track of chip utilization or degradation level. Fig. 3 illustrates the anticipated interactions among the various levels of the system infrastructure.

III. Case Study I: $\mathcal{C}^5$ Adaptation for Error Recovery

The proposed $\mathcal{C}^5$ architecture can be adapted for the purpose of error recovery, in which the correctness of bioassay outcomes is determined by utilizing on-chip detectors located at pre-specified electrodes. In addition, physical-aware control program can be used in a DMFB platform to implement an error-recovery method. The work described in [10] and [11] highlights experimental demonstration for dynamically adapting to error occurrences during assay execution.

As shown in Fig. 4(a), a $\mathcal{C}^5$-compliant, software-based error-recovery methodology relies on a capacitive sensor (at Level I) to examine the appearance of a droplet at a detection cell that is employed as a checkpoint. If a missing droplet is reported via a sensing signal, then a failure must have been encountered during droplet operation. The analog signal is captured via a ring oscillator circuit (at Level II), which, in turn, modulates the sensing signal as a frequency coded signal and forwards it into an FPGA-based frequency divider. At Level III (referred to as the Cyber Level), a microcontroller-based implementation of a firmware is introduced to meter the signal frequency (i.e., collect data), compare the resulting data with a pre-specified error threshold (i.e., analyze data), and communicate the comparison result serially into upper levels hosted on a desktop computer (i.e., report result). When an error occurs, the system reacts by issuing a re-synthesis procedure to recover from the error, taking into consideration that the faulty sites must be bypassed. Ensuring region-

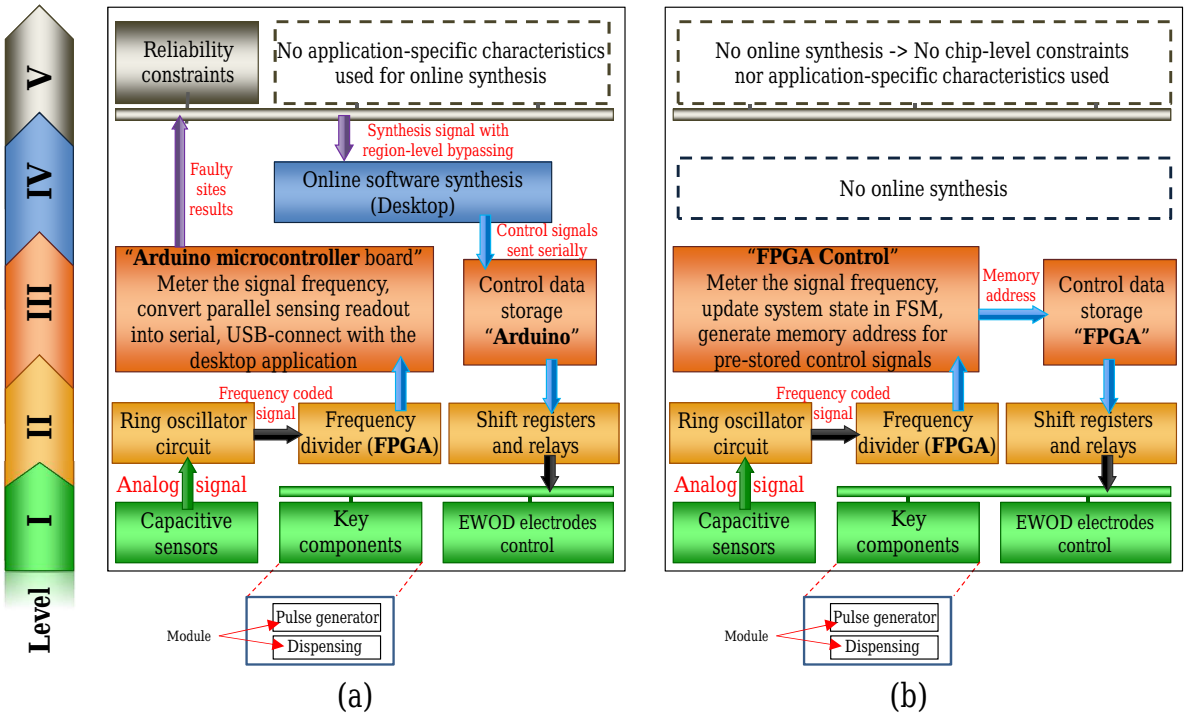

Fig. 4: $\mathcal{C}^5$-compliant architecture design of cyberphysical DMFBs: (a) for software-based error recovery [10], (b) for integrated hardware-based error recovery [11].

level bypassing is accomplished at the Configuration Level (i.e., Level V) to guarantee a reliability-aware re-synthesis at the Cognition Level (i.e., Level IV). After re-synthesis is completed, the resulting actuation signals are communicated back to the microcontroller-hosted firmware (Level III), which triggers low-to-high voltage conversion through an array of relays at the Conversion Level (i.e., Level II), then the biochip electrodes (Level I) are physically actuated.

Similarly, an integrated hardware-based error recovery can be realized using the $\mathcal{C}^5$ concept, as shown in Fig. 4(b). The key-idea of this approach is to pre-compute and store recovery actuation sequences for all errors of interest that can occur during the execution of a bioassay. The detection signal can be used to trigger a "transition" in the system finite state machine (FSM). In other words, when an error is detected, the cyberphysical system simply looks up the recovery solution in the memory that matches the new recovery state, rather than performing online re-synthesis on a desktop computer. This hardware-based solution therefore reduces response time and enables flash chemistry [4], [13].

For this hardware-based solution, the bottom levels (namely Level I and Level II) can be kept unaltered with respect to the previous design (Fig. 4(a)), since sensor data acquisition, conversion, and processing are typically the same in both cases. However, the software Configuration Level (Level V) as well as the Cognition Level (Level IV) are excluded in the hardware-based solution to eliminate the computer-in-the-loop, clearly sacrificing a large degree of system reconfigurability. In conclusion, it is possible that a DMFB design problem, such as error recovery, can be tackled using a $\mathcal{C}^5$-compliant cyberphysical system in different ways; each with its own advantages (e.g., highly reconfigurable vs. highly responsive).

IV. Case Study II: $\mathcal{C}^5$ Adaptation for Gene-Expression Analysis

Recently, protocol miniaturization for gene-expression analysis was realized using DMFBs [12], [14]. On-chip operation begins with the dispensing of sample droplets containing cultured cells. The cells are then lysed in order to obtain intracellular materials (DNAs, RNAs, proteins, etc.). Using magnetic beads, enzymes, and a washing step, mRNA can be isolated and then reverse-transcribed into the corresponding complementary DNA (cDNA) with primers and other reverse-transcription reagents. Next, the resulting cDNA samples are subjected to thermal cycling via qPCR to amplify the target gene.

Designing an autonomous digital-microfluidic system for gene-expression analysis requires the concurrent manipulation of independent samples [12]. Utilizing a set of intermediate decision points, sample-dependent decision-making capability can be incorporated in the cyberphysical system; thus facilitating the manipulation of sample droplets in an unpredicted manner by different bioassays. In addition, the specification of the overall protocol efficiency and the level of gene expression are included on-chip in the feedback system.

To fulfil the aforementioned features, the $\mathcal{C}^5$ methodology assists in constructing the components of cyberphysical DMFBs to support such quantitative protocols. As shown in Fig. 5, Level I includes a variety of on-chip sensors and monitoring systems; each designed for a specific function-

ality. For example, analyzing cell-lysis quality (e.g., assess that samples are not contaminated with bacteria and the cell concentration of the starting samples is acceptable) is accomplished using a CCD camera-based monitoring system, whereas DNA amplification signals are captured by an array of on-chip fluorescence sensors. The Conversion Level (Level II), in turn, is decomposed into partitions; with each partition corresponding to data acquisition, conversion, and encoding from a single sensor. The encoded signal, encapsulated within a "data frame" containing the sensor metadata, is fed into the Cyber Level (Level III) for data processing and analysis.

The Cyber Level (or generally referred to as the firware layer) is especially important for two reasons: (1) it can be deployed on a microcontroller board and/or a desktop computer, depending on the complexity level of data-analysis task. For example, the data frame generated from a CCD camera after completing cell lysis can be processed on a microcontroller board, whereas a desktop computer is preferable for computing gene expression levels after thermal cycling. (2) it is responsible for communicating analysis decisions into the Configuration Level (Level V) so that the procedure flow of a sample pathway can be adjusted. Meanwhile, with a resource-limited DMFB, the deployment of dynamic resource allocation and spatial reconfiguration at Level V is essential and it is motivated by the following realities about biochemistry protocols [12]: (1) the benchtop characteristics of a contemporary microbiology application (i.e., quantitative analysis), where multiple sample pathways are manipulated concurrently and investigated independently (application characteristics; see Fig. 5); (2) the need for a robust design of a DMFB, since a non-robust designs may lead to system failure and inefficient quantification (system performance characteristics; see Fig. 5). While the application characteristics are taken care of by adopting dynamic resource allocation on DMFBs, system constraints are enforced through a spatial-reconfiguration approach. Utilizing both mechanisms in a combined manner ensures a robust miniaturization of a microbiology application on a resource-limited DMFB.

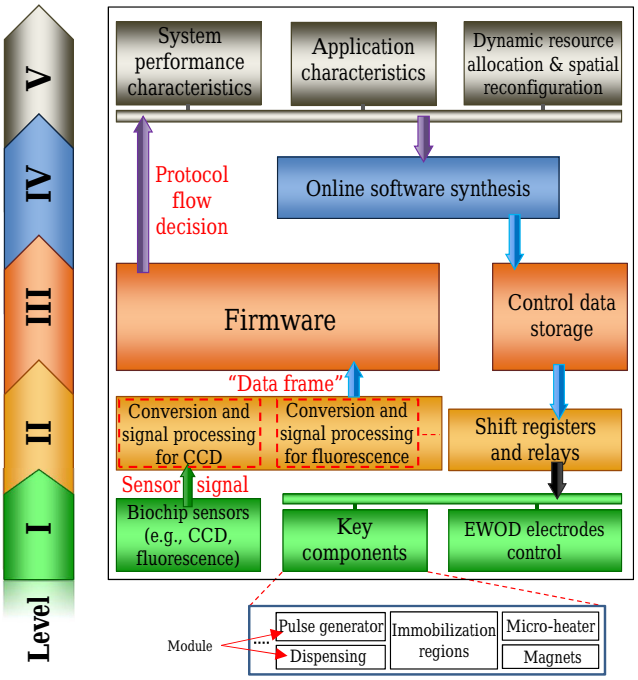

Fig. 5: $\mathcal{C}^5$-compliant architecture design of cyberphysical DMFBs for gene-expression analysis [12].

V. Conclusion

To cope with the complexity of biochemical protocols, we have introduced our vision of a multi-layered architecture for cyberphysical DMFBs. We have presented the functionality of each layer as well as the interplay between these layers. Since the proposed architecture provides a significant reconfiguration capability, we have shown that it can be adapted to various applications including, but not limited to, error recovery and gene-expression analysis.

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