FPGA-based bio-cybernetic system for lab-on-a-chip automation

Kevin I-Kai Wang, Johnny Yeh, and Zoran Salcic
Electrical and Computer Engineering
University of Auckland
Auckland, New Zealand
kevin.wang@auckland.ac.nz

Jin Akagi and Donald Wlodkowic
School of Chemical Science
University of Auckland
Auckland, New Zealand
d.wlodkowic@auckland.ac.nz

Abstract—**In recent years, Lab-on-a-Chip technology has been widely applied to the pharmaceutical and eco-toxicity domains, together with the use of zebrafish as the model organism for performing fish embryo toxicity assay. However, the requirement of constant human attention and lack of fully automated systems have lead into low throughput and slow turnaround time for the experiments. In this paper, a novel FPGA-based bio-cybernetic system is designed to work with Lab-on-a-Chip devices in these experiments for handling zebrafish embryos, controlling chemical liquid perfusion, maintaining micro-environment and acquiring image data periodically for the analysis of embryo development. These functionalities have been demonstrated in the designed system by performing multiple 40-hour continuous experiments.**

Keywords-Lab-on-a-Chip, MEMS, Bio-cybernetic system, FPGA

I. INTRODUCTION

With the advancement of material and fabrication technologies, the state of the art Lab-on-a-Chip (LoC) devices offer attractive features such as mobility, massive parallelisation, small volume of samples and shorter reaction and turnaround time, which are difficult to achieve in traditional chemical and bio-chemical operations. However, the small physical sizes of LoC devices also imply delicate handling procedures and low throughput, especially when intensive human attentions are required. For example, toxicity assay and drug development are expensive, laborious and require constant human attentions, which lead into low throughput and high failure rate. The lack of automation technology has limited the full potentials of LoC in various application domains, including pharmaceutical applications. High throughput automation solutions which enable the experiments and data collection to be performed with massive parallelisation is crucial for drug discovery and toxicology applications in allowing researchers to gain a better understanding on the chemical properties within a short turnaround time. The usage of embedded systems technologies is increasingly expanded to new ranges of applications, notably towards cyber-physical systems, or CPS [1]. In this paper, we present a new class of systems that is beyond the traditional CPS, the Bio Cybernetic Systems (BCS), by extending the CPS and integrating the state of the art embedded technologies with LoC devices to interact with the biological world and live organisms. The target application of this research is the fish embryo toxicity assay (FET) using zebrafish as the model organism.

There are several motivating factors for the work on this practical application. As the technology advances, there are growing concerns of toxic chemical substances and environmental pollution, and hence toxicity assay becomes an important process for many industrial fields, e.g. pharmaceutical and medical industries [2]. Zebrafish is a popular model organism used to perform fish embryo toxicity assay due to reasons such as small physical size, ease of breeding and maintaining in large quantities, short embryo development period, optical transparency for convenient observation and most importantly, the toxic effects observed on zebrafish are closely related to humans [3]. Despite the benefits brought by the zebrafish, the toxicity assay process tends to deal with hundreds of embryos at once and the process may well spread across multiple days, which make the entire process laborious and difficult for constant human attentions. However, it is important to monitor the experiment conditions in order to ensure the reliability. Ideally, each embryo is monitored as an individual and hence the necessity of human operator input becomes a bottleneck to achieve high throughput of the screening process. It is desired to have an integrated platform that combines embedded technologies with micro-electro-mechanical systems (MEMS) and microfluidics technologies into an Automated Biochemical Laboratory (ABL) [4].

Section II of the paper provides an overview of related works in the domain of automating LoC devices. The system overview and requirements are given in Section III, followed by the system design and prototyping in section IV. Section V showcases the evaluation of the developed prototype based on 40-hour trial experiments performed in our lab. Section VI concludes the paper and provides possible future directions based on the current progresses.

II. RELATED WORKS

In recent years, small vertebrate animals, such as zebrafish, were widely used in drug discovery and ecotoxicology applications [2, 3, 5]. Many efforts have been devoted on design the LoC devices that allow easy manipulation of the zebrafish embryos and liquid perfusion [6, 7]. However, as indicated in [8], an integrated platform providing automated specimen loading and sorting, data acquisition, and chemical

perfusion are not yet available. The Complex Object Parametric Analyzer and Sorter (COPAS) [9] is one of the most commonly used automated systems, which is expensive and has a tremendous physical size. Other systems, such as ZebraFactor [10], focus only on sorting the zebrafish embryos using fast imaging techniques. Small portable devices that make use of the LoC technology are more commonly seen in point-of-care diagnostic devices [11] or medical diagnostic devices [12], rather than in laboratory-based microfluidic experiments which handle small live organisms.

While most of the researches focus on the design of the LoC devices, the goal of this research is to develop a versatile FPGA-based platform which automates most of the experiment procedures to achieve high throughput and low turnaround time for the FET applications. The platform is designed to automatically load embryos into the LoC device; control liquid perfusion; maintain the micro-environment within the device; and periodically record image data for observing the process of embryo development.

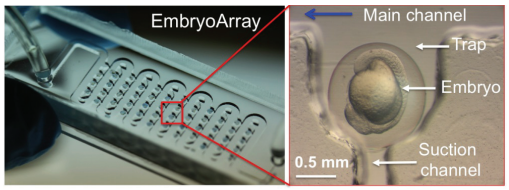


Figure 1. Microfluidic zebrafish embryo array for fish embryo toxicity (FET) assay.

III. System Specifications

In this paper, the target LoC device is designed for trapping zebrafish embryos for chemical toxicity assay applications, as depicted in Fig. 1 [13, 14]. The first reduced functionality prototype of the fully automated system was developed and presented in [4]. It was made as a functional prototype based on the use of a standard microprocessor and off-the-shelf components. In this paper we present an FPGA-based extended implementation that integrates all critical hardware and software components of the system in a single FPGA chip and leads towards an industrial scale full size ABL with high throughput.

In order to design an automated embedded system for this target application, the requirements are specified and presented in the following sub-sections. Fig. 2 illustrates the system overview of the proposed ABL platform, which consists of a number of microfluidic valves, pumps, thermal sensor and thermoelectric, a camera and a LoC device. The platform can accommodate multiple LoC devices, which means additional sensors and actuators are also needed.

A. *Fluid channel selection*

One of the key requirements of automating LoC devices is to precisely control the fluid flow within the overall system. The flow can be controlled by using two types of components, microfluidic pumps and valves. Referring to Fig. 2, the fluid perfusion and flow rate is controlled using the flow control pump. The other function of the pump is to provide suction force that immobilises the embryos into each of the traps within the LoC device. The flow rate must be precisely controlled at a very low speed, typically less than 2ml per minute. Multiple valves are used to select different routes of liquid flow under different experiment modes. For example, during the embryo loading process, flow control valve 3 is opened to allow embryos to enter into the LoC device. Valves 1 and 2 are used to regulate toxic chemicals and water flow respectively at different stages of the experiment.

B. *Temperature regulation*

Due to the fact that zebrafish is a tropical freshwater fish, different properties of the micro-environment within the LoC device, such as liquid temperature, pH level, and oxygen level is very important to successfully hatch the embryos. With proper flow rate control, the oxygen and pH level of normal tap water is sufficient for hatching the embryos, whereas the water temperature can be greatly affected by the ambient temperature. Therefore, the fluid temperature must be strictly regulated to be between 28°C-29°C.

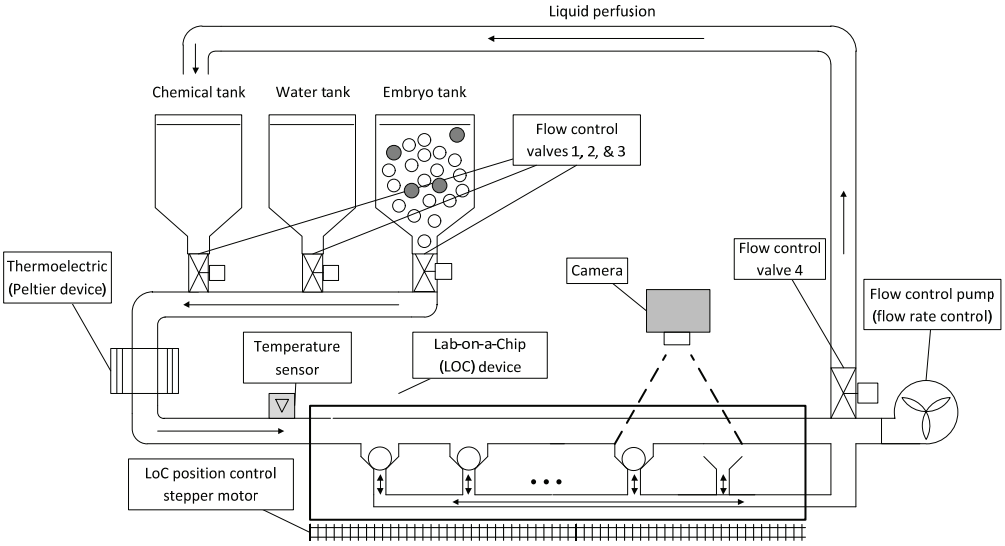


Figure 2. Automated Biochemical Laboratory overview.

C. Automatic image acquisition

In order to achieve high throughput screening, the system must reduce human involved operations within the experiments to the minimum amount. Automatic image acquisition should be performed periodically. In the target application, an image of each embryo should be collected every 30 minutes. In addition to the automatic image acquisition, real-time image analysis would also help in improving the throughput and turnaround time of the experiment by monitoring and reporting the results immediately after experiment termination or even during experiment when needed.

D. Chip positioning

As mentioned previously, an ABL platform may contain one or more LoC devices that hold hundreds of embryos. Each embryo development process should be observed and recorded by the camera periodically. While the camera is in the fixed place, the LoC device can be moved in one or two dimensions to place each embryo within the camera view for automatic image acquisition. The LoC positioning process must be synchronised with the image acquisition process.

TABLE I. REQUIRED TIMERS AND IO PINS FOR THE EMBEDDED PLATFORM.

Embedded Devices	1-channel LoC			5-channel LoC		
	No. of device	Timer	IO pins	No. of device	Timer	IO pins
Stepper motor pump	1	1	4	5	5	20
Chemical valve	1	0	1	5	0	5
Embryo valve	1	0	1	1	0	1
Water valve	1	0	1	1	0	1
LoC positioning motor	1	1	4	2	2	8
Sensor SPI bus input	1	0	4	1	0	4
Thermal-electrics (Peltier)	1	1	2	1	1	2
LED camera lighting	1	1	1	1	1	1
Count		5	20		10	44

IV. SYSTEM DESIGN AND PROTOTYPING

Following the system specifications in section III, each embedded platform contains one stepper motor pump for flow rate control; a solenoid valve for each of the chemical, embryo and water container; a LoC positioning stepper motor; temperature sensor input on the serial SPI bus; a Peltier device for temperature regulation; and LEDs for camera lighting. Each of these devices occupies certain amount of I/O pins and maybe a timer for generating the control signal. Referring to Table 1, the number of timer units needed for generating the control signals (i.e. PWM signals) poses a major constraint on the embedded controller. The previous embedded functional prototype presented in [4] used an Atmel AVR-based microcontroller with very limited capability for extension and no capability to control multi-channel LoC due to limited hardware resources (e.g. number of timer units which are critical for this type of system). In comparison with an off-the-shelf microcontroller, a FPGA chip, such as the Altera Cyclone II FPGA family, can support up to 622 IO pins and customised number of timer units in the FPGA, which provides a more flexible solution. In the following subsections, the FPGA-based embedded controller and the external temperature regulation circuit are explained in details.

A. FPGA-based embedded controller

A System-on-a-Programmable-Chip (SoPC) solution that includes the soft-core NIOS II processor and various peripheral components are implemented in the Altera Cyclone II FPGA chip. Referring to Fig. 3, The NIOS II processor interfaces with the peripheral components through the Avalon switch fabric. The internal EPCS flash memory is used to provide static program memory, while the SDRAM and on-chip RAM are memories available for the system at runtime. The NIOS II processor and IP components operate with a 100 MHz system clock. This system clock is slowed down to 1 MHz for the need of many electro-mechanical components such as pumps and stepper motors. The use of FPGA allows creation of multiple PWM output modules controlling various actuators.

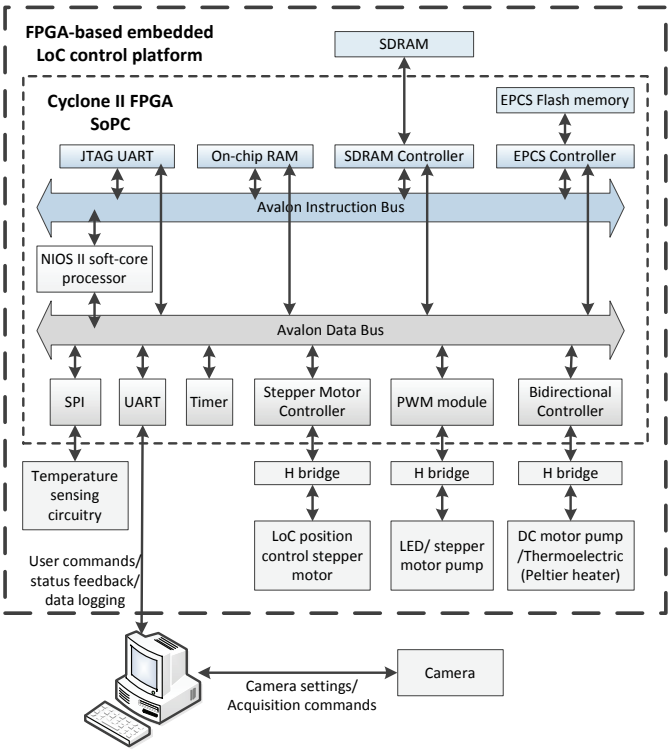


Figure 3. Architecture of FPGA-based LoC control platform.

Referring to Fig. 3, besides the already provided IP components, including SPI, UART and Timer modules, three customised modules, namely stepper motor controller, PWM module and bi-directional controller are designed for controlling the used actuators. These customised control modules are discussed in the following subsections.

1) PWM module

The LEDs lighting source of the camera can be controlled using PWM signals where the duty cycle of the PWM signal determines the light intensity. Also, the drive circuit that

controls the stepper motor pump varies the flow rate according to the frequency of the PWM control signal. Both actuators are controlled by their own PWM module that can change the frequency and duty cycle of the output square wave controlled by a program.

This module contains a counter that constantly counts up in response to a clock tick (based on a pre-scaled clock). The value of the counter is reset according to the user-configured value of clock divider. This determines the frequency of the output square pulse. The user-configured duty cycle value determines at which point during a period the output switches from high to low.

2) Bi-directional controller module

This module is used to generate a PWM signal that controls a two terminal device. In the ABL system, this device can be a DC motor pump or a thermoelectric (Peltier) heater. Voltages across these devices can be applied in both directions to reverse device behaviour (i.e. clockwise/anti-clockwise rotation or heating/cooling).

The module has two signal outputs that control the driving circuit made of an H-bridge, which in turn powers the two terminals of an actuator. The first output is a PWM signal similar to the PWM module with adjustable frequency and duty cycle. The second output is a constant logic zero. The role of these two outputs can be switched by writing to the direction register.

3) Stepper motor controller module

This module generates the four phase drive signals for controlling a single stepper motor. Stepper motors are used to control the LoC device position with high precision. The module has two main modes of operation. Firstly, the continuous mode cycles through the drive signals to cause the stepper motor to rotate continuously. The speed and direction of rotation is controlled via the clock divider and direction registers, respectively.

The second mode allows the controller to cause the motor to rotate through a discrete number of steps. In addition to controlling the rotation speed and the direction, a steps register determines the number of steps the motor will rotate before stopping. When the number of remaining steps becomes zero, the module outputs an interrupt request signal (IRQ). The programmer need to ensure the steps register is not written again before the previously requested number of steps is completed and the IRQ signal emitted. This ensures the final LoC device position is the same as the anticipated position determined by the number of steps written to the register.

B. Temperature regulation unit

The micro-environment has a great influence on embryonic development. Therefore, it is crucial that factors such as temperature, pH and oxygen levels are precisely controlled during experiments to ensure valid and reliable results. Referring to Fig. 4, a close loop temperature control system was developed to regulate the temperature of chemical fluid being delivered to the embryos. As the FPGA chip does not have an analogue to digital (A/D) converter, an external A/D converter chip, MPC3304, is selected to provide the conversion. It is a 13-bit differential A/D converter that communicates through SPI interface. The SPI controller is added to the NIOS II processor for interfacing with the A/D converter to retrieve the temperature sensor measurements. Since FPGAs operate in LVTTL (3.3V), a step up voltage translator is required to interface with peripheral electronics that functions in LVCMOS (5V), as shown in Fig. 4. The MC14504B voltage translator is selected for this purpose.

1) Temperature monitoring

Temperature monitoring can be approached by three different types of temperature sensors: thermocouples, resistance temperature detectors (RTDs) and thermistors. In order to achieve precise temperature monitoring for the target application, investigation has been done to compare all three types of thermal sensors. Thermocouples produce voltage variations in the order of tens of microvolts corresponding to the change of 1^{o}C and hence require an extremely high precision amplifier in order to read the temperature measurement at the resolution required. Also, thermocouples are more commonly used for measuring the differential temperature between the two terminals of the sensor, rather than the absolute temperature needed for the target application. Different to the thermocouples, the resistance of RTDs reflects the absolute temperature measured. However, RTDs are generally applied in systems that measure over a wide range of temperatures with lower resolutions.

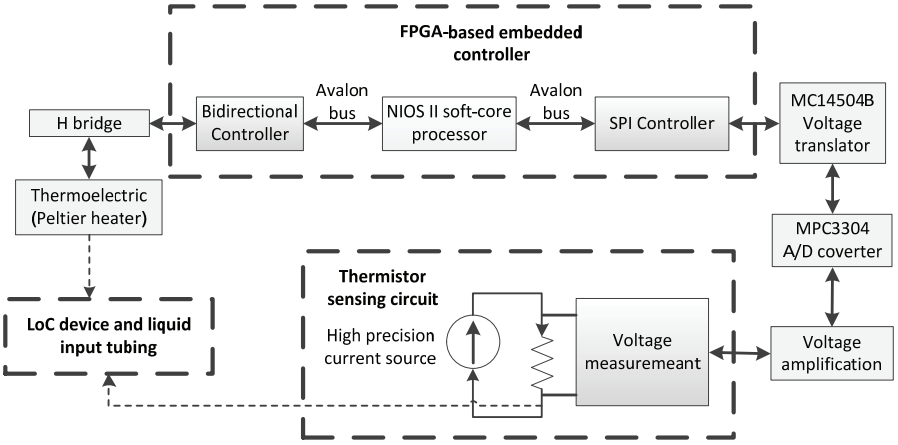


Figure 4. Temperature regulation unit overview.

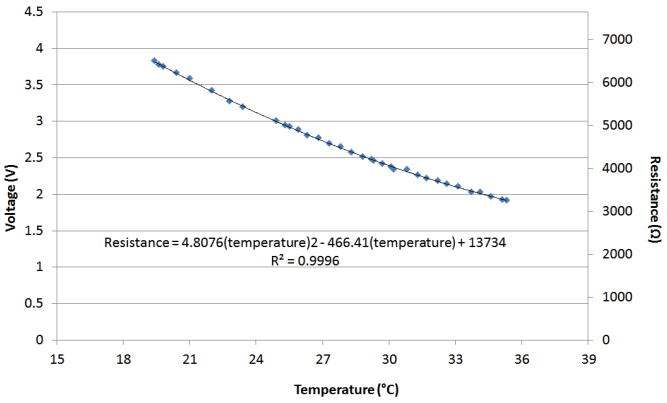


Figure 5. Calibrated resistance-temperature relationship of the selected thermistor.

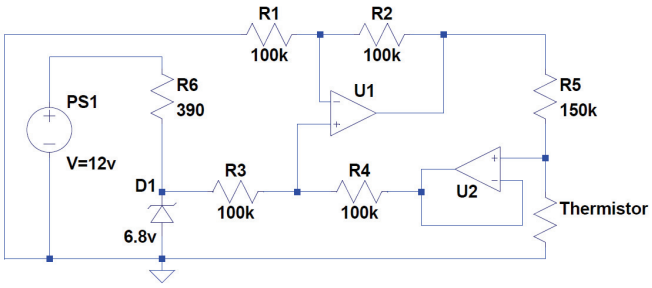


Figure 6. High precision current source for thermistor operation.

Thermistors are similar to RTDs in that their resistances change according to the absolute temperature. They have larger resistances compared to RTDs, allowing a greater voltage signal to be obtained across the sensor, which significantly simplifies the measuring circuit. Compared to RTDs, thermistors usually work in a smaller temperature range and are much more sensitive to temperature variations, which is suitable for the application in this project. The thermistor used is an NTC (negative temperature coefficient) type, which means the resistance decreases when temperature rises. In general, thermistors have a non-linear resistance-temperature relationship and curve fitting is necessary to determine the measured temperature. The selected thermistor works in the range -40°C to 125°C and has a tolerance of 0.5°C. The resistance-temperature relationship was calibrated using a water bath and the resistance-temperature curve is presented in Fig. 5.

Referring to Fig. 4 and Fig. 6, a high precision current supply was used to power the thermistor. The current through the thermistor was 45 µA, which allows approximately 2.9 V to be measured across the thermistor at 25°C after amplification, with the voltage varying approximately 0.1 V/°C. With an external 13-bit A/D converter (resolution of 1.22 mV), the temperature sensor can achieve a theoretical resolution of approximately 0.01°C. The resolution will vary at different temperature due to the nonlinear characteristic. The A/D converter readings were converted into the linear temperature scale using the polynomial equation in Fig. 5. A measuring resolution of 0.1°C can be achieved by calibrating individual thermistors. However, better tolerance than 0.5°C is required to achieve this resolution across different thermistors.

A. Temperature control

A thermoelectric (Peltier) device was chosen as the actuator of the temperature regulation unit. It has the advantage of being able to actively heat or cool its surface. When a voltage is applied to the device, a temperature gradient is created between the hot and cold side of the device (i.e. differential temperature rather than absolute temperature). Larger temperature gradient is created when a larger voltage is being applied. Reversing the voltage direction reverses the hot and cold side of the device, which enables both heating and cooling operations. In order to achieve absolute temperature control, one side must be fixed at the ambient room temperature by attaching a block of aluminium to the Peltier module with thermal paste, which acts as heat sink to maintain this reference side at the room temperature. Different temperature gradient can then be applied to actively control the absolute temperature on the other side.

An H-bridge IC (SN754410, Texas Instruments) was used to drive the Peltier device. This allows voltage across the device to be applied in either direction, hence allowing active heating or cooling of the delivered fluid. The bi-directional controller module in the embedded FPGA controller outputs the PWM signal for controlling the Peltier heater. Through altering the duty cycle, the size of the temperature gradient can be controlled precisely by the embedded FPGA controller.

The Peltier device has a low resistance of ~1.7 Ω. Therefore, the device needs to be powered at a low voltage. The 12 V power supply on the ABL prototype system was stepped down to 5 V using a voltage regulator. Two Peltier devices were connected in series to increase the overall resistance. With this configuration, the current drawn by the device is between 0.5 A to 1 A, which can be safely supplied by the H-bridge IC. Temperatures between 15°C to 35°C can be obtained on the controlled side of the device. During the initial trial experiment, a PI controller with the Peltier device was implemented and the surface temperature of the device was relatively constant, varying within 0.5°C after the temperature stabilises.

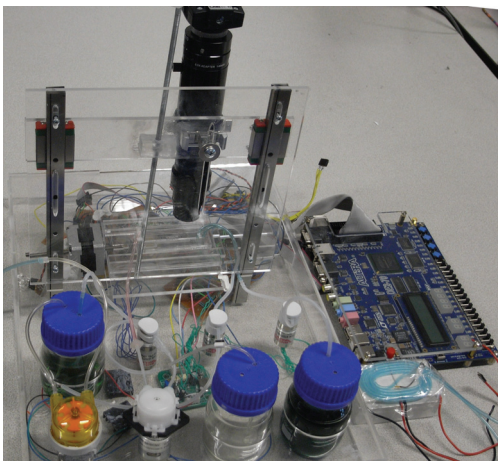


Figure 7. Prototype of the proposed ABL platform.

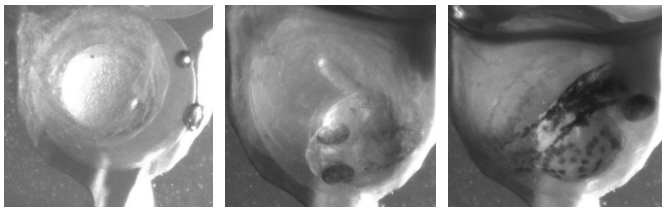
V. PROTOTYPE EVALUATION

Based on the proposed system, a prototype of the ABL platform has been implemented as shown in Fig. 7. A trial FET

assay experiment has been conducted for over 40 hours to demonstrate the ability of the designed platform to fulfil all the system requirements mentioned in section III: 1) accurate flow control for loading embryos and carrying out the experiment; 2) maintaining the micro-environment such that zebrafish embryos can properly hatch; and 3) automatic image acquisition which allows later observation of the embryo development process.

As mentioned in section III, the target micro-environment control in this prototype is the temperature of the flowing liquid, which must be maintained at 28°C±0.5°C for the zebrafish embryos to hatch successfully. Fig. 8 shows three images acquired automatically during the experiment every five minutes for offline analysis. The first image (Fig. 8(a)) presents the embryo at 0 hour and has no sign of development. In Fig. 8(b), the eyes of zebrafish larva can be observed from the image, which indicates the embryo has developed for more than a day after fertilisation. The last image shows pigmentation stage of the skin of the larva, which starts happening roughly after 40 hours of the embryo fertilisation.

Based on the trial experiment, the prototype platform has demonstrated the ability to fulfil all the design requirements, including properly controlled flows to load embryos and circulate chemical fluid; precisely controlled flow rate to trap the embryos while providing sufficient liquid flow to maintain oxygen level; accurate temperature regulation in allowing zebrafish embryos to hatch successfully; and automatically acquire images for offline observation and analysis. The final FPGA controller designed on the Cyclone II chip uses less than 20K logic elements (LEs), which is 28% of the LEs of the entire chip. This indicates the solution has high flexibility and extensibility in supporting additional sensing and actuating requirements if needed. This represents a much more compact and flexible solution compared to the initial prototype in [4].



(a) 0 hour (b) 30 hours (c) 40 hours

Figure 8. Zebrafish embryo development recorded on the prototype platform over a 40 hours trial experiment.

VI. CONCLUSION AND FUTURE WORKS

In this paper, a novel FPGA-based bio-cybernetic system has been designed to automate the experiment process of zebrafish embryo toxicity assay. The system is able to 1) automate the process of loading embryos into the LoC device by precisely controlling the flow rate; 2) maintain the liquid temperature within the LoC device in a range of 28°C-29°C for the zebrafish embryos to hatch; 3) periodically acquire images which record embryo development process for later analysis. The implemented prototype has been evaluated by performing 40-hour experiments and has demonstrated the ability to perform all the required functionalities. The solution is highly efficient and is flexible for accommodating more sensing and actuating requirements.

A number of future research works has been planned based on the current progress. An image analysis tool is currently under development and will be integrated with the system to provide real-time monitoring and analysis, which allows better throughput and shorter turnaround time. Also, an automatic embryo sorter and feeder has been designed to feed one embryo into the LoC device at user specified frequency. Other sensors, such as the pH sensor, are also considered in the next system prototype to provide better status information of the micro-environment within the LoC device.

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