WIRELESS CLOSED-LOOP CONTROL OF CENTRIFUGO-PNEUMATIC VALVING TOWARDS LARGE-SCALE MICROFLUIDIC PROCESS INTEGRATION

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ABSTRACT

We present for the first time an integrated wirelessly powered Arduino processor and Bluetooth interface that are co-rotated with the cartridge to allow large-scale process integration on a centrifugal microfluidic platform. This highly modular, electronically controlled "Lab-on-a-Disc" system (eLoaD) can independently actuate up to 128 normally-closed valves by an array of microheaters during rotation for comprehensive, highly parallelized sample-to-answer automation. Additionally, we implement real-time optical (colour intensity) measurement for closed-loop control of liquid handling, sample preparation and detection.

INTRODUCTION

Over the recent decade, centrifugal microfluidic platforms have been of increasing interest for decentralised bioanalytical testing such as point-of-care diagnostics [1]. The microfluidic cartridges typically share the form factor of commonly available optical discs such as CDs or DVDs. Fluidic functionality is enabled by rotating the cartridge by a simple, and often low-cost, spindle motor, thus obviating the need for a costly and potentially error-prone pump. 'World-to-chip' interfacing is a key advantage of the LoaD platform. Where most other platforms require pressurized fittings and custom sealing and priming processes, the LoaD samples can be loaded at atmospheric pressure using a simple pipette. Their inherent capability to centrifuge samples is extremely useful for Laboratory Unit Operations (LUOs) for blood processing and particle / cell handling constitutes a further advantage of LoaD systems.

However, as the centrifugal field acts on all liquids on the disc simultaneously, flow control elements, such as valves operate in a highly reliable manner to permit automation of complex processes involving a sequence of LUOs.

Broadly speaking, valving schemes on the centrifugal platform can be categorized into externally actuated and rotationally controlled schemes. Common, normally-closed rotationally actuated valves open upon varying the spin rate. This type of valve is typically based on unbalancing the hydrostatic equilibrium between rotationally induced pressure and the other forces acting on liquid elements such as pneumatic (counter) pressure or the capillary action; thus the key advantage is the only control input required is modulation of the spin rate. This category comprises capillary burst valves, siphons and dissolvable film barrier [2,3].

In the case of externally actuated valves, a peripheral instrument (other than the platform-innate spindle motor) transfers energy to the disc, e.g. via thermal energy, kinetic energy, electrical energy and mechanical force [2].

Platforms enabled by such externally actuated valves typically offer greater levels of integration density compared with the rotationally actuated valves; however, this often comes at the expense of increased cost and complexity. Yet, the decreasing cost and improved ease-of-use of micro-components has resulted in the use of such platforms becoming more common.

In this work, we combine the previously introduced 'electrified Lab-on-a-Disc' (eLoaD) [4] platform with our event-triggered dissolvable film (DF) valves [2] to actuate up to 128 valves in random sequence. In the conventional implementation, the event-triggered valves are composed of two dissolvable films, called the load film (LF) and control film (CF), placed in a dead-end pneumatic chamber. Venting trapped air from the pneumatic chamber through dissolving the CF permits the liquid to contact the LF and continue via the LF orifice. This implementation, which is analogous to a single use electrical relay, permits a number of complex implementation including use of Boolean control logic (i.e. AND and OR control logic).

Recently, we presented a version of the event-triggered valves where the CF was replaced by a pierceable membrane. By scouring the top of the disc with a robotic knife-blade, we could actuate valves during rotation [2]. Here, rather than piercing the membrane with a knife-blade, we use a gas-tight, low-melting temperature membrane (commonly available Parafilm) as our CF. Upon melting at ~60ºC, the membrane deforms away from the temperature source and thus leaves the resistive heaters intact.

A key advantage of this platform is that wireless data and power transfer, being enabled via a commonly available commercial phone-charger [4], obviates the need for modification of the motor spindle, e.g. by ancillary wiring or an electrically rather noisy slip-ring connector; effectively the controller is aligned with the disc, placed on the spindle motor, secured in place and the phone charger is positioned in proximity to the receiver coil (Figure 1).

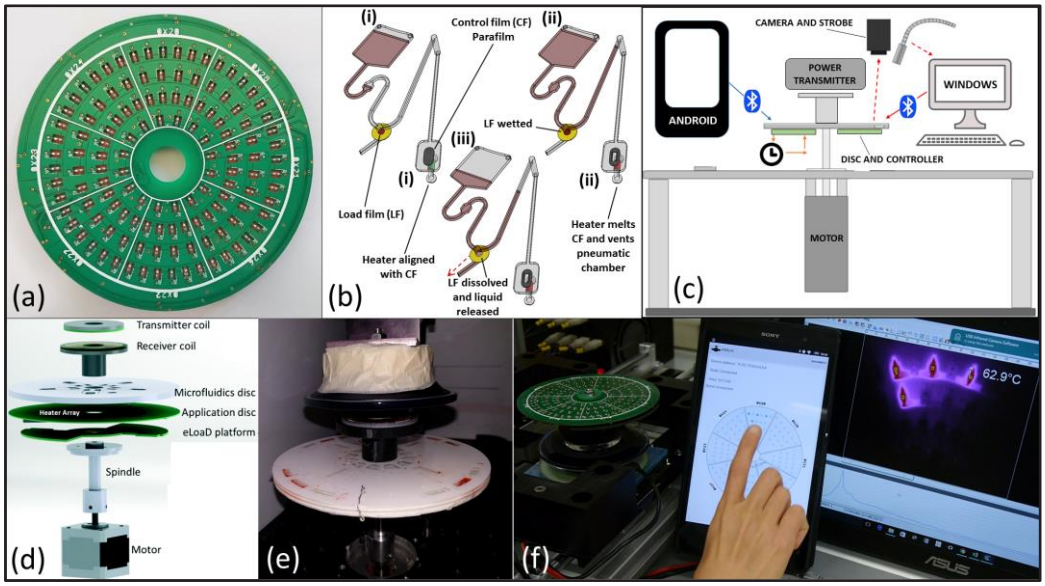

Figure 1: (a) Disc featuring 128 wirelessly powered and controlled microheaters for independent actuation of the normally-closed valves. (b) Valve operation and (c) platform concept with the three control options – internal timing, manual control via an Android app and closed-loop control or manual operation via a Windows PC. (d) Exploded view of the platform. (e) Assembled system with a commercial phone charger (transmitter coil) mounted above the motor unit. (f) In the IR thermography, the hot resistors correspond to those manually activated via the Android app

In a first control method of the eLoaD platform, an internal script can be run (initiated either by a Bluetooth command or simply by providing power to the microcontroller) which imposes a well-defined and accurately timed sequence of valve actuations. In the second, manual control method, commands are directly issued by a custom app which can run on common Android-based tablets via a Bluetooth module. We further developed a Windows-based LabVIEW program for manual or script control of the heaters. Finally, this code was integrated with motor control and image analysis software to permit real-time closed-loop control of LUOs.

To demonstrate the capabilities of this wirelessly powered platform, we show colour-based closed-loop image analysis to mix dyed with salt water though 'shake-mode', i.e. Euler-force mixing. We then demonstrate blood processing whereby, through the use of density gradient media, we isolate plasma and white blood cells from a whole blood sample.

EXPERIMENTAL METHODS

Disc Manufacture and heater array

The discs used in this study were manufactured using xurography [3] in a multilayer architecture from layers of Poly(methyl methacrylate) (PMMA) and layers of Pressure Sensitive Adhesive (PSA).

Microchannels were created from voids in PSA using a knife-cutter (CraftRobo Pro, Graphtec, USA) while larger reservoir structures were laser cut (Epilog Zing, USA) in 1.5-mm thick PMMA discs. To facilitate the Parafilm (low melting temperature film), the layer configuration previously described [3] was adapted:

1. Loading and vent layer – 0.5 mm PMMA
2. Upper microchannel layer – PSA
3. Reservoir layer – 1.5 mm PMMA
4. DF cover layer – PSA
5. DF support layer – PSA
6. First middle layer – 0.5 mm PMMA
7. Middle microchannel layer – PSA
8. Second middle layer – 0.5 mm PMMA
9. Lower microchannel layer – PSA
10. Base (vented) layer – 0.5 mm PMMA
11. Parafilm support layer – PSA
12. Parafilm layer – Parafilm

The DFs are prepared by mounted on PSA to improve sealing and to make them mechanically stable during assembly [3]. Pneumatic venting channels were machined in the middle and lower microchannel layers to allow routing of any valve on the disc to any thermal heater. Venting of the valves was facilitated by vertical vias located in the base layer. These valves were then sealed by a layer of Parafilm which was adhered to the base using the PSA.

The disc was mated to the microchannel heater array by alignment pins such that each heater lined up with the venting vias located in the base layer. Upon heating, the Parafilm quickly melts, thus venting the valves (to atmosphere). Typically, the Parafilm deforms away from the heat source and therefore the heater array did not require any cleaning between use.

The heater array was based on the modular, wirelessly powered system previously elucidated [4]. This Arduino based system enables individual heaters to be activated, via Bluetooth command, in an arbitrary sequence. The power is sufficient for heating up to 10 heaters simultaneously.

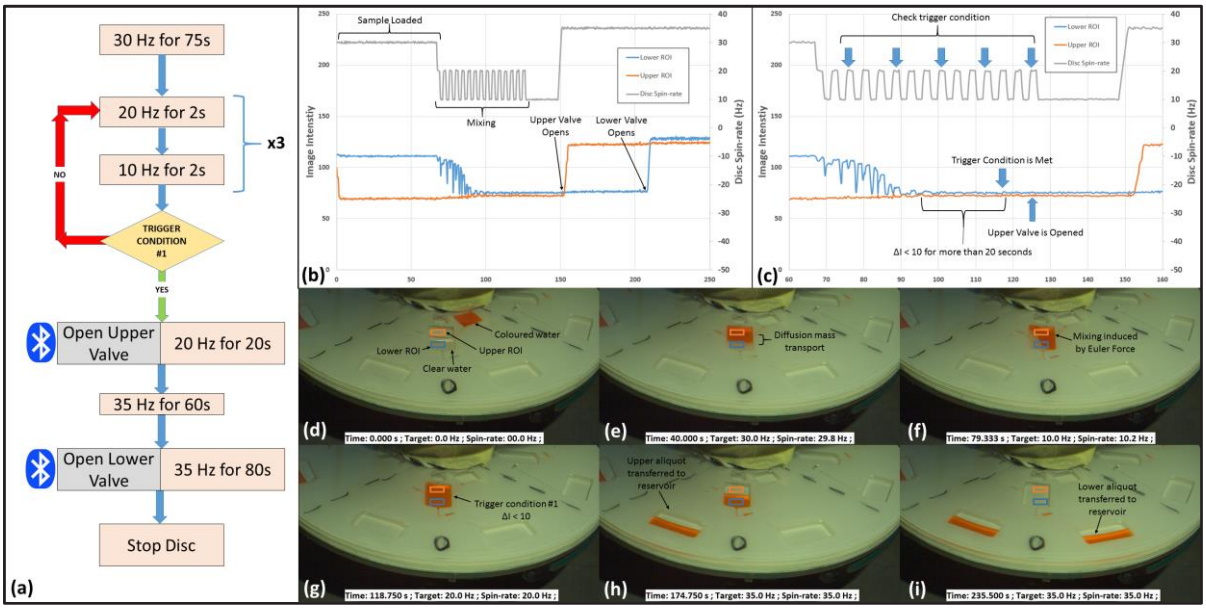

Figure 2: Closed-loop control of mixing. (a) Programme decision tree. (b-c) Spin protocol and intensity plots for the entire experiment. (d) Detail of the system meeting Trigger Condition #1. The trigger was found in a YES state after 5 mixing cycles. (d) Disc as dye is loaded (e) Diffusion mass transfer during the first steady-state spinning (f) Convective mixing induced by Euler forces ('shake-mode' mixing). This convection is reflected in the downward spikes in intensity within the lower ROI during mixing. (g) Chamber after completion of mixing. (h) Upper valve opened. (i) Lower valve opened.

Experimental Test Stand and Real-time Data Analysis

During testing, images are acquired using an experimental 'spin-stand' composed of a high-powered motor (Festo EMME-AS-55-M-LS-TS, Esslingen, Germany) synchronised with a strobe (Drelloscop 3244, Drello, Germany) and a sensitive, short-exposure time camera (Basler Ace 2040-90uc, Basler, Germany) using custom hardware acquiring 5 frames per second.

Real-time image analysis is enabled by acquiring and saving images from the Basler Ace camera using Basler Pylon software and saved this to a folder on the PC hard-drive in bitmap format. A custom LabVIEW code is used to control the experiments. This code has three components parts; the Bluetooth communication module, the motor control module and the image analysis module. During operation, the code continuously monitors the image acquisition folder and loads into memory the most recently created bitmap image. Images are typically loaded within 100 ms of their creation, thus resulting in quasi real-time monitoring of the camera output.

Prior to the start of the test, the user defines several Regions of Interest (ROIs) on the disc, each of them associated with colour intensity based triggers. The conditions can include the ROI exceed or dropping below an arbitrary intensity level (useful for process monitoring) or the intensities in two ROIs approaching each other (useful for monitoring mixing).

EXPERIMENTAL RESULTS

Closed-loop fluidic mixing

In order to demonstrate closed-loop control of on disc processes, we applied our system to on-disc mixing. Due to the prevalence of laminar conditions in microfluidic system, mixing poses a challenge. In centrifugal microfluidics, mixing is typically enhanced through use of 'shake-mode' mixing; for this the disc is rapidly accelerated so the resulting Euler force agitates advection within the liquids. Here, we implement real-time monitoring for tracking the convergence of colour intensity of two ROIs (Figure 2). The mixing is initiated by one of the wireless control modes.

To present this work, we used a disc which had an approximately rectangular mixing chamber. This chamber has a valve part way down the side of the chamber and one at the base of the chamber. We first load the disc with DI water and centrifuge so the water fills the lower half of the mixing chamber. Next, two ROIs are defined, one above the liquid level and one below the liquid level. The disc is then loaded with dyed water (1% concentration of common red food dye) and the test is started. As the disc is accelerated, the red dye enters the mixing chamber where it is over-layered on the transparent water.

As can be seen in Figure 2, the colour intensity of the upper ROI decreases but the colour intensity of the lower ROI stays largely steady. Mass transport via diffusion starts to occur but, over the first 75 seconds of the test, overall mixing is negligible. When 'shake mode' is initiated, convective mixing occurs and this is reflected in the rapid decrease in colour intensity in the lower ROI. After approximately 60 s, the colour intensities in the two ROIs converge and the pre-defined trigger condition (that the difference in intensities is less than 10 units for 20 consecutive seconds). Meeting this trigger condition results in the program leaving the mixing phase and opening the two consecutive valves in a timed sequence.

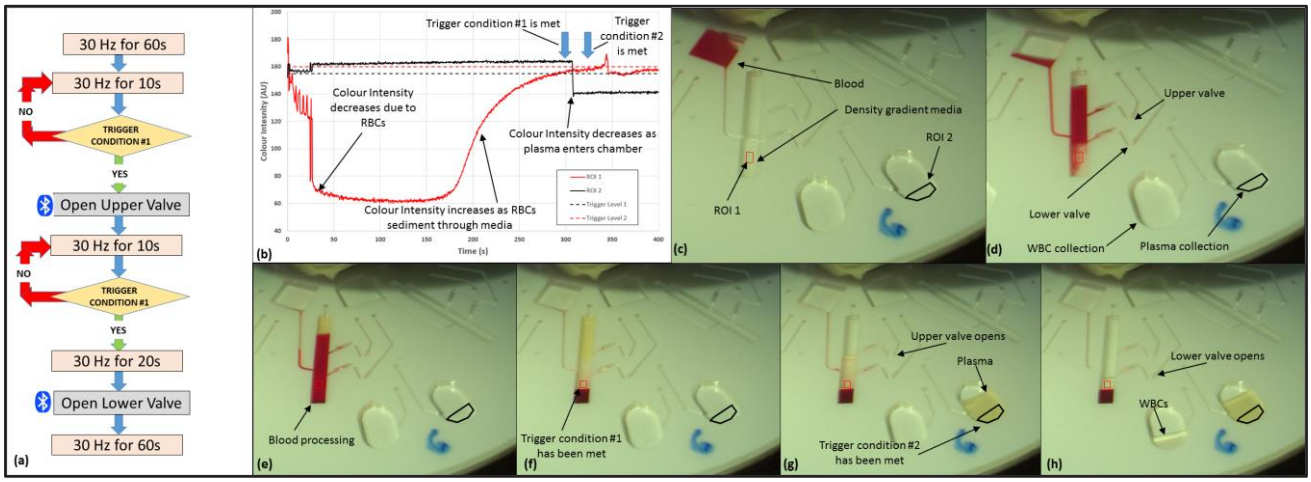

Figure 3: Closed-loop control of blood processing. (a) Programme decision tree with two triggers. Trigger Condition #1: Colour intensity in ROI 1 exceeds a cut off value (155 AU for 20 s unbroken) where this indicates that most erythrocytes have sedimented through the density-gradient medium. Trigger Condition #2: Colour intensity drops below 160 AU (for 20 s unbroken) in ROI 2 – this indicates the entry of plasma into the plasma collection chamber. (b) Spin protocol and intensity plots for the entire experiment. Note that the colour of the intensity plots matches the ROIs (c-h). Blood processing is indicated by a drop in colour intensity in ROI and its subsequent recovery. Entry of plasma into the plasma collection chamber is indicated by a decrease in colour intensity in ROI. 2 (c) initialisation of blood processing (d-e) and ongoing blood processing. (f) Blood processing is deemed completed as the colour intensity in the ROI exceeds the cut-off value. (g) Upper valve opened to remove plasma (i) and lower valve opened to remove buffy coat.

Closed-loop blood processing

We next demonstrate closed loop control of blood processing. Compared with other microfluidic systems, the centrifugal platform is highly suited to blood processing and this is a common sample preparation step in a number of assay protocols. Here, we use density gradient media to extract both plasma and white blood cell from a whole blood. Initially we extract the blood from a finger prick sample using a protocol described previously. We dilute these samples 1:1 with a dilution buffer using a protocol described previously [5]. To process the blood, we first load 40 µl of density gradient media (DGM) (Histopaque 1077, Sigma-Aldrich) onto the disc and centrifuge at 30 Hz; this volume is choses so the liquid reaches the level of our inlet channel. We then load 80 µl of diluted whole blood onto the disc. The test program is then started and, as described in Figure 3, red blood cell (RBC) sedimentation is monitored and the separation process fully controlled.

CONCLUSION

In this paper we have introduced a novel, electronically controlled valving mechanism for the centrifugal "Lab-on-a-Disc" platform whereby valves are actuated by activating a resistive heater which co-rotates with the disc. This "eLoaD" platform is enabled by a wirelessly powered micro-controller and heater array. The system has a fast response time of approximately 20 seconds and can enable actuation of an unprecedented number of valves. Significantly, the system is very light and does not require electrical wiring between units on the rotor and stator; thus, eLoaD is compatible with very low-cost motors and can be readily integrated into portable systems. Unlike many instrument actuated systems, the platform can permit valve actuation during rotation.

ACKNOWLEDGEMENTS

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