

XUROGRAPHY ACTUATED VALVING FOR ARBITRARY TIMING OF CENTRIFUGAL FLOW CONTROL IN PARALLELIZED MULTI-STEP BIOASSAYS

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ABSTRACT

Here we introduce a new, instrument controlled valving scheme for the centrifugal platform which is based upon dissolvable film (DF) technology. Liquid, restrained at any point upon the disc, is prevented from wetting a DF via a trapped gas pocket. From this pocket a pneumatic channel runs to a sealed vent located on the top surface of the disc. Controlled scouring of this seal by a robotic knife-cutter permits venting of the trapped gas, and thus actuation of the valve. To demonstrate the potential of these valves, we present a disc developed towards a biplex liver assay panel.

KEYWORDS: Xurography, Centrifugal Microfluidics, Dissolvable Films

INTRODUCTION

Over the recent decade centrifugal microfluidic systems have increasingly entered the arena of biomedical point-of-care diagnostics [1]. In these systems, a typically disc-shaped cartridge is rotated at defined frequency protocol by a robust and low-cost spindle motor. This simple actuation scheme renders these so-called ‘‘Lab-on-a-Disc’’ (LoaD) devices independent of external pressure sources and their pneumatic connectors, thus ensuring ease-of-use and minimum maintenance. This work significantly advances a centrifugo-pneumatic valving scheme by a stage-mounted blade to perforate a sacrificial membrane. We demonstrate the automation of multi-liquid, multi-step sample preparation and reagent handling which are key ingredients towards large-scale integration and automation of bio-analytical assays. Critically for on-disc blood processing, the valves are externally actuated during rotation, thus ensuring efficient plasma extraction.

Centrifugal flow control is predominantly implemented by changes in the spin rate [2]. For instance, in capillary burst valves, the centrifugally induced hydrostatic pressure overcomes the counteracting capillary pressure imposed by a hydrophobic constriction. Apart from the system-innate spindle motor, flow control can also be achieved by additional, instrument-based modules, for example by laser ablating a sacrificial membrane or by heating a phase-change material.

Recently, Kinahan *et al.* [3,4] introduced ‘event-triggered’ flow control based on dissolvable-film (DF) membranes. These valves are composed of a dead-end pneumatic chamber sealed by two DFs.

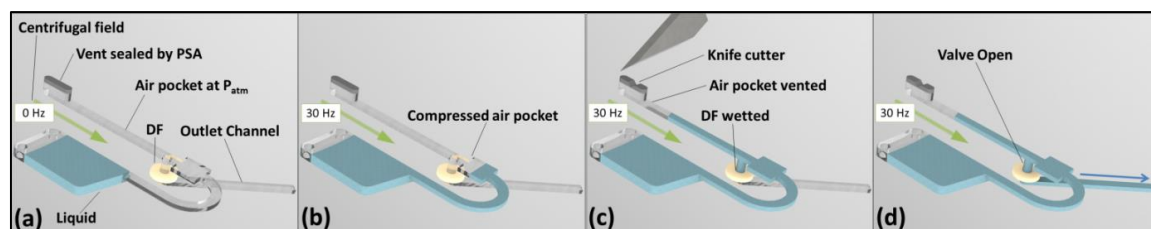


Figure 1: Operation of the xurography-enabled DF valve. (a) The valve consists of a pneumatic chamber with two outlets closed by a DF tab and PSA. (b) The gas pocket trapped between the meniscus of the incoming liquid and these outlets initially prevents the wetting of the DF. (c) To open the valve, a knife blade mounted on a robotic arm (Fig. 2) scours the top of the disc, and thereby piercing the PSA. (d) As the gas pocket decompresses, the DF is wetted and dissolves to clear the passage of liquid to the outlet.

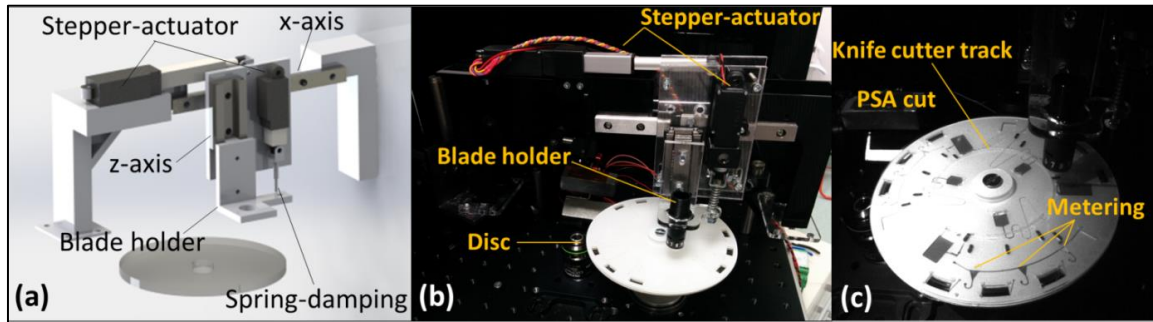


Figure 2: Robotic arm. (a) 3D rendering. The plastic components are either 3D printed or laser-cut and assembled using adhesive. Low-cost slide rails provide mechanical support while the translational stage is actuated by low cost stepper motor (Firgelli). The actuators, which provide positional feedback, are controlled in our setup by a custom LabVIEW program with data acquisition card. (b) The robotic arm mounted on spin-stand. The liver assay disc (Fig. 3) is mounted on the experimental rig. (c) Monochrome image acquired while the disc is spinning. The image is acquired using an angled camera (to view both liquid movement and the knife cutter) by synchronizing the camera / strobe with the spin-stand motor. The disc is loaded with red dyed water for visualization purposes. The track of knife cutter track can be seen via the piercing of the PSA layer to vent and open Valve 1.

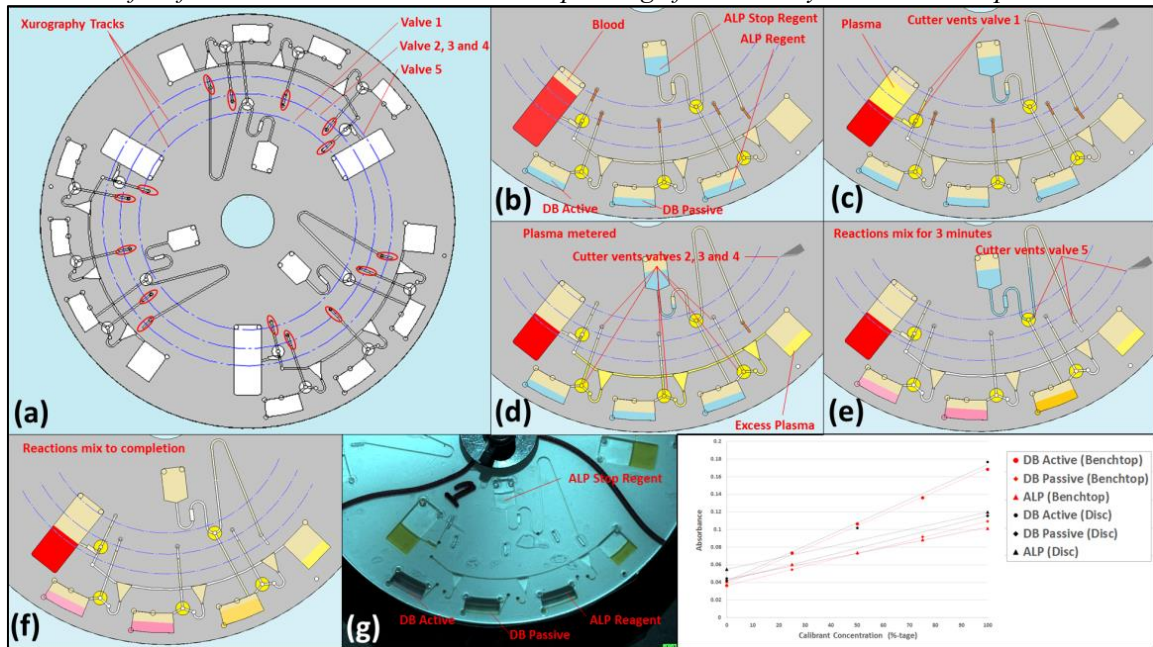


Figure 3: The Lab-on-a-Disc for a biphase liver assay panel measuring Direct Bilirubin (DB) and Alkaline Phosphatase (ALP). While the test for DB runs in an endpoint format, a reagent has to be added after 3 minutes to stop the ALP reaction. A DB control (passive DB) calibrates the active DB reaction. (a) The disc displays three separate structures which are operated in parallel. (b) A blood sample is centrifuged on disc (the same structure can be loaded with DI water or calibrant) (c) The knife cutter opens Valve 1, releasing the plasma to be discretized and metered. (d) The knife cutter opens Valve 2, 3 and 4, thus releasing the metered plasma (10 μ l) to mix with reagents. (e) After 3 minutes, the knife cutter opens Valve 5 to release the reagent stopping the ALP assay. (f) The reactions run to completion. Note that, at all times, the disc is subject to Euler-force based mixing. (g) Image of the disc just prior to opening of Valve 5. (h) Comparison of calibration data acquired on benchtop and on-disc (using dilutions of supplied calibrant). This data is acquired from dilutions of supplied reagent calibrant. The reagent protocol recommends a two-point calibration curve. A single disc can generate calibration curves using two structures (0% and 100% calibrant) and run a patient sample in the third structure.

Upon removal of the so-called control film (CF), the pneumatic chamber is vented to let the liquid escape by dissolving the so-called the load film (LF). Such event-triggered architectures have shown to automate an unprecedented number of individual liquid handling steps in a single disc at an essentially arbitrary, e.g. constant spin rate. However, a major drawback of such event-triggered valving is its poor flexibility of timing, e.g. for biochemical reactions, which is quite rigidly governed by the dissolution time of the DF and the passage time of the liquid between assay steps.

Based on an adaptation of the event-triggered valve architecture, we introduce in this work significantly enhanced control of timing by xurography; (Fig. 1). In this case the CF is replaced by a vent, sealed by adhesive tape, which is located on the top surface of the disc. A blade, adapted from a commercial knife cutter, is mounted on a 2-axis robotic arm which positioned above a disc (Fig. 2). During rotation, the blade is lowered to perforate the adhesive tape to vent the pneumatic chamber. By locating the vents at the different or the same radial locations, valves can be opened in parallel, or in a defined sequence at prescribed times.

EXPERIMENTAL

A robotic arm is manufactured via 3D printing and / or laser cutting as shown in Figure 2. This module is automated using low cost robotics and controlled via a custom LabVIEW program. The arm is then mounted over a stroboscopic ‘spin stand’. Discs are manufactured using polymer lamination methods as previously described [3,4]. To seal the pneumatic vents the gas tight adhesive tape is rolled onto the entire upper surface of the disc. Loading holes and the central spindle hole are then cut out manually.

RESULTS AND DISCUSSION

To demonstrate timing of flow control with the novel, xurography-actuated flow control scheme, we implemented a multi-step bplex liver assay panel [5] (direct bilirubin and alkaline phosphatase) with integrated sample calibration. This way three discrete liquid handling steps, i.e., blood separation, plasma metering (opening three valves in parallel) and addition of a stop reagent to ALP have been successfully orchestrated with precise timings (Fig. 3). Towards eventual measurements with blood samples, we test this disc with supplied calibrant and compare results to benchtop controls (Fig. 3h).

CONCLUSIONS

This novel flow-control mechanism permits enhanced temporal control of the lab on a disc. As their actuation is now decoupled from both, the spin rate of the disc and dissolution time of the DF, the valves can be opened at arbitrary times and in arbitrary sequence. The positioning of multiple vents on the same radius allows parallel actuation of valves to enable concurrent processing of samples. This flow control scheme thus shows promise towards large-scale integration of bioassays.

ACKNOWLEDGEMENTS

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