BUOYANCY-DRIVEN CENTRIPETAL PUMPING FOR NESTED SAMPLE PREPARATION IN BIOASSAYS

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ABSTRACT

In this paper we present a method of buoyancy-driven centripetal pumping utilizing a heavy, water-immiscible liquid to displace an aqueous sample radially inwards against the prevailing centrifugal force. Using formerly introduced dissolvable-film valving, we demonstrate metering and bidirectional periodical pumping of a single sample in the radial direction. We then show integrated sample discretisation, metering and buoyancy-driven inward pumping in a microfluidic structure designed towards multi-parameter bioassays. This structure permits centrifugation of sample on the periphery of a disc (where the centrifugal field p) and proves a high degree of metering accuracy better than 1.2%.

KEYWORDS: Lab-on-a-Disc; Centripetal Pumping; Displacement; Metering

INTRODUCTION

Centrifugal microfluidic systems have shown particular aptitude for applications in the field of point-of-care biomedical diagnostics, particularly where ruggedness, portability and cost efficiency are key [1]. These "lab-on-a-disc" (LoaD) platforms are advantageous for engineering low-complexity instrumentation as rotational flow control only requires actuation by a spindle motor.

However, a significant downside of the LoaD system is the unidirectional nature of the centrifugal field. The limited radial of the disc confines the number of laboratory unit operations (LUOs) such as metering and mixing that can be integrated onto a LoaD cartridge. Furthermore, upstream sample preparation processes, such as blood centrifugation, must run at rather central locations, thus necessitating elevated spin rates to generate the required centrifugal field. Similarly, reagents must also be stored near the centre of the disc where real estate is inherently limited.

To mitigate these limitations, a number of methods for centripetally directed pumping have been developed. In addition to the basic spindle motor, modules have been employed to provide energy sources such as compressed air [2] as well as thermally [3] or electrolytically [4] induced volume changes. Centrifugo-pneumatic pumping involving rapid rotational acceleration and deceleration [5] as well as positive [6] and negative [7] liquid displacement-based pumping has been demonstrated. While efficient, these methods require complex sample loading procedures which may not be practical in certain applications.

We introduce here buoyancy-driven centripetal pumping which uses (water) dissolvable films (DFs) [8] to enable accurate centripetal pumping of metered liquid volumes. Beyond state-of-the-art, displacement-based pumping schemes, DF-based flow control brings in several merits. In particular, it is demonstrated that a single sample can be repeatedly pumped radially inwards and outwards without

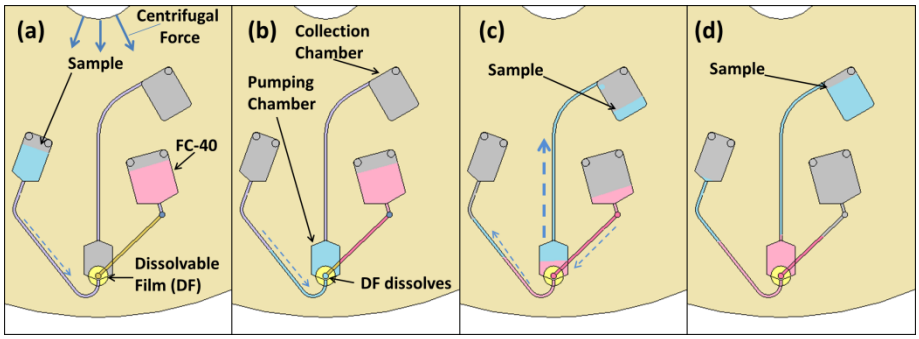

Figure 1 – Auto-triggered displacement pumping using DFs on a multi-layer disc (a) initial disc configuration (b) sample in pumping chamber (c) denser FC-40 liquid displaces sample radially inwards (d) centripetal pumping of sample completed.

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external intervention as the flow is triggered by the mere presence of the liquid at a defined location. Furthermore, with the view to implementation of a multi-parameter bio-assay [9], we demonstrate the capability to centrifuge a sample located on the periphery of a disc (thus necessitating lower spin rates) followed by an aliquoting step into uniformly metered volumes.

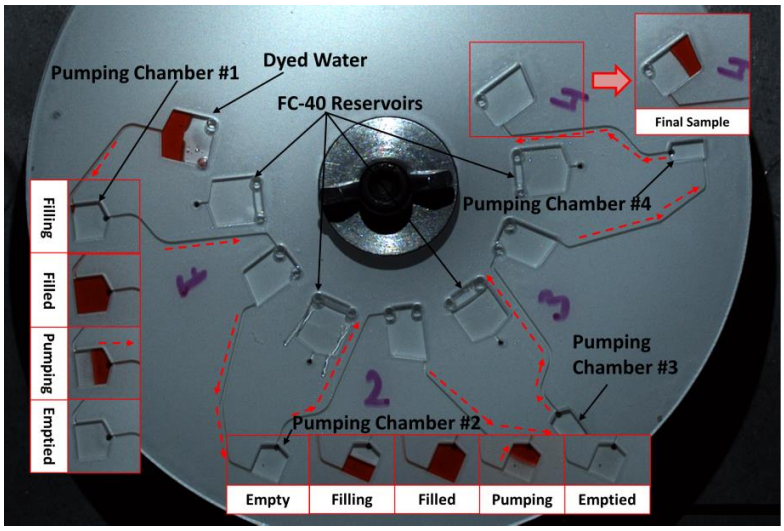

Figure 2 - Four-fold centripetal displacement pumping of an aqueous sample on a disc rotating at a constant spin rate.

PUMPING MECHANISM

As detailed in Figure 1, a water-immiscible liquid FC-40 fluorocarbon (Sigma Aldrich) exhibiting a specific weight of 1.85 is stored proximal to the centre. The solvent-selective DF (HarkePro, Germany) stays intact against the FC-40 while slowly dissolving within ~50 s in contact with water. This DF is placed in the pumping chamber to initially seal the microchannel (located on the lower layer of the disc) which is connected to the FC-40 reservoir (Fig. 1a).

The contact of the aqueous sample dissolves the DF (Fig. 1b), thus prompting the release of FC-40. Due to its immiscibility with water and its higher density, FC-40 then centripetally displaces the aqueous sample. The cross sections of the pumping chamber and the microchannel as well the metered liquid volumes are chosen in a way that the sample is pumped against the centrifugal field into the central collection chamber (Fig. 1c).

EXPERIMENTAL

The discs are manufactured by a method previously described multi-lamination methods [8].

DF-triggered flow control is first demonstrated by 4-fold bidirectional pumping of a liquid sample. Note that the entire protocol is run at constant spin rate (here: 30 Hz) and that each pumping step is solely triggered by the arrival of liquid in the pneumatically connected chamber. First, the sample is centrifugally driven into the peripheral Pumping Chamber #1 (Fig. 2). Note that frames 3 and 4 in each film strip are taken from the same video stills, thus showing that when one reservoir is partially empty the next is partially filled. The final location of the sample after passing through nine chambers is shown the inset image in the top left.

We then extend this method to a structure which can process whole blood and discretize the extracted plasma into three symmetric aliquots while pumping them radially inward. This structure, which is showcased here using food dyes, is geared towards integrating a 3-parameter liver assay panel on whole blood [9] followed by an automatically triggered calibration step.

At the start, sample is loaded in a processing chamber and is centrifuged at a low spin rate (< 30 Hz) to mimic plasma extraction (Fig. 3a). The spin rate is then increased to 30 Hz for opening the DF-based burst valve (Fig. 3b) and thus pumping a metered volume of dyed water ("plasma") radially outward. Here, the sample flows into a metering chamber and opens the DF that so far restrained the immiscible (Fig. 3c). Consequently, this heavier liquid is automatically released and the sample is displaced upwards into structures designed to aliquot 24-µl volumes (Fig. 3d-e). An event-triggered valve [8] then automatically triggers the identical process in an additional calibration structure (Fig. 3f).

The biomimetic sample is pumped into reaction chambers which have been pre-loaded with a known, 120-µl volume of DI water. The absorbance of the mixture of unknown volume of food dye with a defined volume of DI water is measured and, using a standard curve (inset of Fig. 3f), the volume of metered in each structure is assessed. These measurements (n = 9) indicate accurate and repeatable metering of 24.4 µl ± 280 nl.

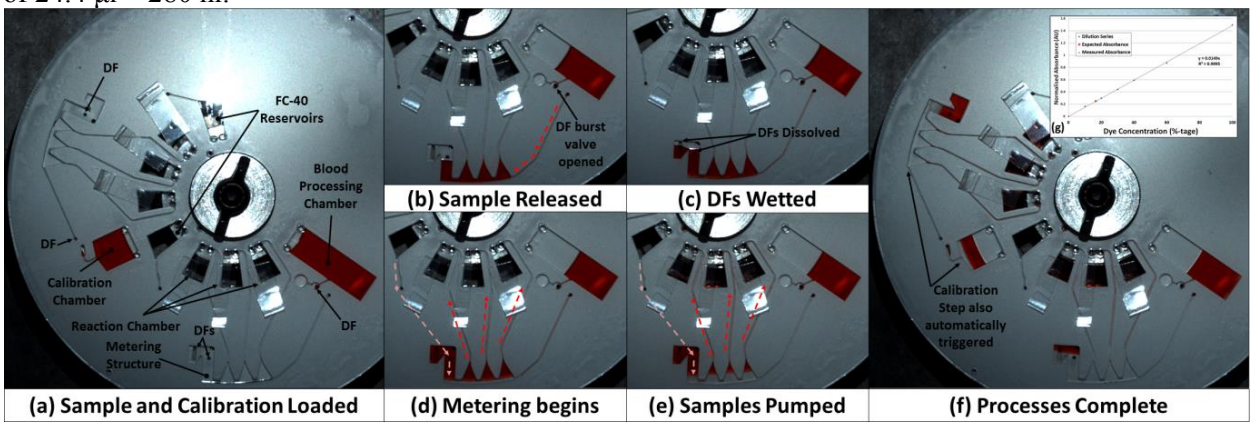

Figure 3 - Combined centripetal pumping and metering in a structure with an automatic calibration step.

CONCLUSION

Our novel centripetal pumping method makes efficient use of real estate by allowing to place larger, upstream (sample preparation and reagent storage structures) in the outer, more spacious regions on the disc cartridge. The event-triggered technique, which can be implemented in a reciprocating manner, also provides accurate metering and aliquoting functions which exhibit minimal dead volumes compared with some equivalent valving technologies. Thus this technology bears great potential to set the stage for reliable automation of parallelized bioassay panels through actuation by a simple spindle motor [9].

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