µFLU14-38

RECIPROCATING, BUOYANCY-DRIVEN RADIAL PUMPING ON CENTRIFUGAL MICROFLUIDIC PLATFORMS

Niamh A. Kilcawley, David J. Kinahan, David P. Kernan, Jens Ducrée*
[1] Biomedical Diagnostics Institute, National Centre for Sensor Research,
School of Physical Sciences, Dublin City University; Ireland
niamh.kilcawley3@mail.dcu.ie, david.kinahan@dcu.ie,
david.kernan5@mail.dcu.ie, jens.ducree@dcu.ie

KEY WORDS

Centripetal Pumping, Centrifugal Microfluidics, Multiphase Liquids, Metering, Bio-Assay,

ABSTRACT

Centrifugal microfluidic systems bear great potential for applications where ruggedness, portability, ease-of-use, and cost efficiency are critical. However, due to the unidirectional nature of the centrifugal pumping force, the number of sequential process steps which can be integrated on these "Lab-on-a-Disc" (LoaD) devices is limited by their finite radial extension. To significantly widen this bottleneck and thus expand the scope of applications that can be ported on these LoaD platforms, various groups have developed a range of centripetal pumping mechanisms. Here, we present two advancements over our previous efforts in this area by combining buoyancy-based pumping with dissolvable film (DF) valves. First, we present a buoyancy-driven, reciprocating flow of a dense liquid initially located an upper reservoir and a sample in a peripheral reservoir. Secondly, we combine buoyancy-driven centripetal pumping with sample discretization and metering to fully integrate and automate a liquid handling protocol towards implementing a multi-parameter bioassay on a disc.

INTRODUCTION

On LoaD platforms [1-2], liquid handling protocols are integrated and automated on a cartridge typically exhibiting dimensions similar to disc-shaped optical data storage media (e.g. CD or DVD). These centrifugal microfluidic systems offer conceptually simple actuation of liquid handling within a disposable cartridge free of pneumatic interfacing; furthermore, the centrifugal field and thus the sedimentation and pumping pressure can be varied over orders of magnitude as they scale with the square of the spin rate set by a simple spindle motor. This resulting ruggedness, cost-efficiency, autonomy and portability of the platform make the LoaD platform a promising candidate for bioanalytical assay automation in low-resource settings, e.g. for point-of-care testing in the doctor's office or even at the patient's home, in-field environmental monitoring [4], at-line bioprocess monitoring [5] and global diagnostics [6].

However, the unidirectional nature of the centrifugal field tends to contradict transport of liquid from the periphery to the center of rotation. Thus, owing to its finite radial extension, discrete Laboratory Unit Operations (LUOs) [4] such as mixing and metering must be placed serially along the radial direction. On the one hand, its finite radial extension restricts the number of LUOs that can be integrated on a disc. Secondly, initial sample preparation steps such as centrifugation and reagent reservoirs must necessarily be located at a central location where disc space as well as the sedimentation force are minimal.

In order to make optimum use of precious real estate near the axis of rotation and to maximize locally induced centrifugal force required for initial sedimentation steps, a repertoire of centripetal pumping methods has been developed. In a common approach, external support instrumentation directly or indirectly creates a pressure head to pump liquid centripetally against the prevailing centrifugal force. These ancillary energy

sources include compressed air bottles [8], heaters to expand trapped gas [9] and electric power driving on-disc electrolysis [10]. While useful, these methods may compromise the inherently simple actuation of LoaD systems by a conventional spindle motor. Therefore entirely spindle driven solutions have been implemented such as rapid ramping of the spin rate pressurises a gas pocket which expands during subsequent deceleration has been implemented for centripetal pumping[11]. However, the efficiency of this technique relies on the relative flow resistances of the inlet and outlet channels and also the use of a powerful motor to run the steep ramps of the rate of rotation.

Alongside approaches using gas expansion, liquid displacement pumping has also been demonstrated. In a positive displacement approach [12], a pumping liquid is stored near the centre of the disc. Upon release into an elongated chamber, this liquid moves the working fluid radially inwards. Through a variety of shaped chambers, energy is transferred between liquids using two methods. In one approach, an intermediary gas was compressed by displacement of a pumping liquid towards the periphery of the disc; expansion of the compressed gas then drives the sample liquid towards the centre. In the second approach, the immiscible pumping liquid (carbon tetrachloride, specific gravity (SG) ~1.6) is released to flow underneath the sample and to displaced in the radially inbound direction. A similar approach, based on negative displacement [13], was presented where an intermediary gas is used as a 'microfluidic pulley' to move liquid from the periphery to the centre of the disc. However, in either concept, the discs require laborious / complex sample loading procedures and sealing to create trapped gas pockets while the use capillary burst valves limits the range of operational spin frequencies.

Recently, we introduced a buoyancy-driven pumping scheme [14] which combined (water) dissolvable films (DFs) [15-16] with a heavy liquid. This scheme enables accurate centripetal pumping of defined, metered volumes in a user-friendly manner which is facilitated through use of a heavy, immiscible, low-viscosity liquid (FC-40 fluorocarbon, SG ~1.85) which will not dissolve the DF. FC-40 is stored in a pumping chamber proximal to the center behind a slow-dissolve DF (~50 s). The addition of liquid to a reservoir on the edge of the disc (called a lower pumping chamber), where the DF is located, releases the immiscible liquid which then flows radially outward, thus displacing the sample liquid radially inward.

In this work we present two significant advancement of our previously established, buoyancy-driven pumping methods. In the first case, we improve the efficiency (both hydraulic and use of disc real-estate) of our pumping mechanism by establishing a reciprocating flow; as the sample liquid is pumped radially inwards it is directed into the upper pumping chamber and is overlaid on the dense FC-40. Thus the sample increases the pressure head generated by the FC-40 during pumping while it is also stored in the same chamber. A second DF, exposed to the aqueous sample as the FC-40 level drops, then dissolves and releases the sample to flow radially outward, for instance to be subject of further LUOs.

In a second improvement, we expand our previously established metering / pumping structure [14] through the addition sample mixing chambers sealed by DF burst valves [15]. The addition of these chambers allows us to demonstrate a structure which can be used to implement a multi-parameter bio-assay for monitoring liver function [17].

2. CARTRIDGE MANUFACTURE AND METHODS

2.2 Disc Manufacture and Assembly

The microfluidic cartridges used in this study were assembled four layers of Poly(methyl methacrylate) (PMMA) and four layers of PSA (Pressure Sensitive Adhesive, Adhesives Research, Limerick, Ireland) by a previously described multi-lamination method (Fig. 1) [16]. Microchannels and other small features were created from voids cut in PSA (86 µm thick) [18] using a knife-cutter (Graphtec, Yokohama, Japan). Larger features such as reservoirs were laser cut (Epilog Zing, USA) into the PMMA layers (1.5 mm thick). These eight layers were stacked as follows:

1. Layer 1 (PMMA) consisted of loading vents.
2. Layer 2 (PSA) consisted of top-level microchannels for liquid transport and pneumatic venting.
3. Layer 3 (PMMA) provided large reservoirs and through-holes which act as vertical vias.
4. Layer 4 (PSA) was a cover layer which sandwiches the DF tabs in place.
5. Layer 5 (PSA) was a support layer for DF tabs and contained voids where the DF tabs could be positioned.
6. Layer 6 (PMMA) contained the through-holes which acted as vertical vias.
7. Layer 7 (PSA) features the lower-level microchannels for guiding flow and pneumatic venting.
8. Layer 8 (PMMA) acts as mechanical backing.

As they are isolated from each other, the microchannels on Layers 2 and 7 could cross and thus enable higher-level complexity.

The layers were manually aligned on a custom assembly jig and subsequent hot-roll lamination pressing (Hot Roll Laminator, Chemsultant Int., US). Previously prepared DF tabs (See Section 2.2) were placed in voids present in Layer 5 and covered by Layer 4 for mechanical reinforcement and proper sealing.

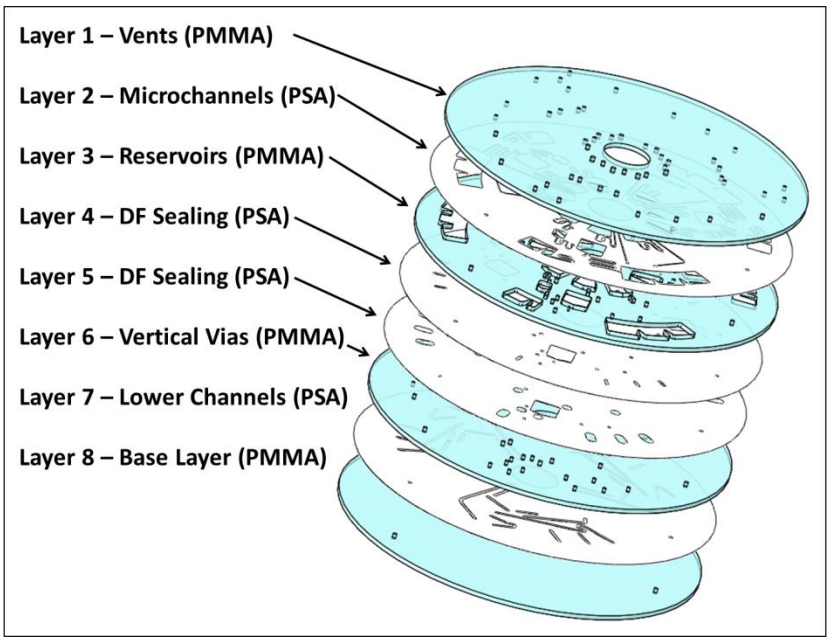

Figure 1: Detail of disc assembly from eight layers which are stacked into a multi-layer architecture.

2.2 Dissolvable Film Tabs

As they are non-adhesive, DFs were attached to double-sided pressure sensitive adhesive (PSA, Adhesives Research, Limerick, Ireland) in order to create sticky tabs [15-16]. The PSA is backed by an upper and lower carrier layer. First, a standard paper cutter machine (CraftRobo Pro, Graphtec, USA) cuts through-holes into the upper carrier layer and the PSA layer. The PSA chads, along with the entire upper carrier layer, are then removed and the DF material is rolled onto the exposed adhesive. A second cut, through the DF material and PSA, then defines the outline of the tabs. The outer diameter of the tabs was defined as 3 mm and the inner diameter 1 mm.

During assembly, the tabs can be peeled from the lower carrier layer. The tabs were produced in larger-number arrays for use in the here described cartridges. Sealed in gas-tight bags, they could be stored for subsequent manufacturing runs. Note also that two grades of DF are used - both based on a polyvinyl alcohol (PVA) chemistry. Quick-dissolve, low-cost DFs, called Embroidery Film (Barnyards, Rippon, UK) were used for centrifugo-pneumatic burst valves while a slow-dissolve valve (~50s, Solublon KC-35, Harkepro, Germany) [16] were used to restrain the pumping liquid (FC-40).

2.3 Experimental Test Stand

As the discs must be tested while under rotation, they are characterized using an experimental test platform commonly referred to as a "spin stand" [19] which has been described previously [16]. A computer controlled motor (Faulhaber Minimotor SA, Switzerland) rotates the discs at defined rates. The motor encoder is synchronized using custom hardware with a stroboscopic light source (Drelloscop 3244, Drello, Germany) and a sensitive, short-exposure time camera (Pixelfly, PCO, Germany). Due to the stroboscopic principle, a stationary image of the same disc section is obtained for each rotation. Aside from where otherwise specified, all discs are tested at 30 Hz rate of rotation. The discs are accelerated and slowed down at 12.5 Hz s^{-1}. Images are acquired as single files (PCO's propriety format) at a rate of ~6 Hz.

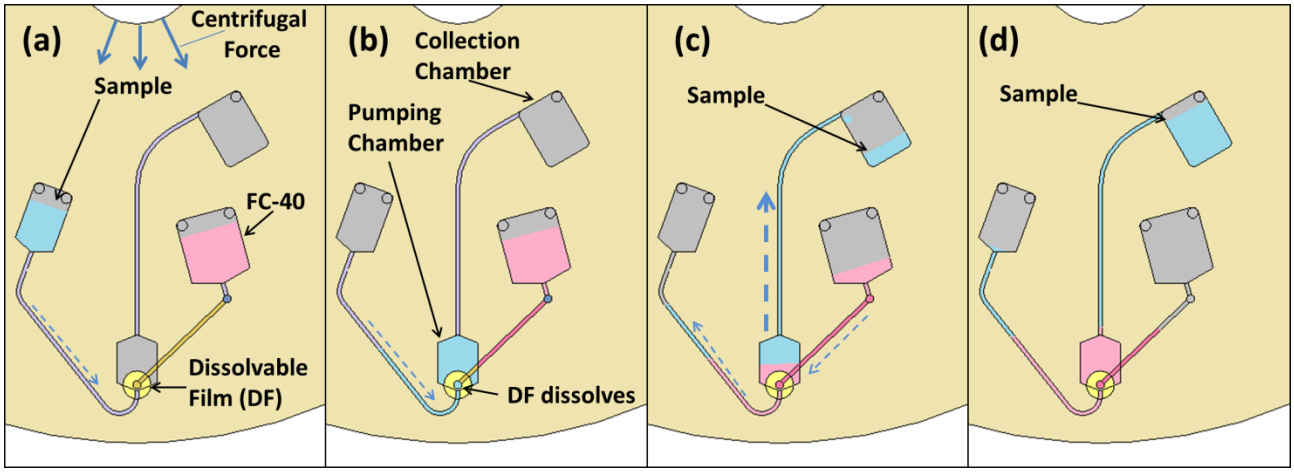

Figure 2: Basic Principle of Buoyancy Driven Centripetal Pumping (adapted from [14]). (a) Disc cartridge in the initial configuration. The FC-40 is restrained by the DF (b) the sample is pumped into the pumping chamber and the DF dissolves. (c) FC-40 is released and displaces a defined volume of sample radially inwards into the collection chamber (d) the sample is now located radially inwards.

3. MECHANISM OF BUOYANCY-FRIVEN PUMPING

The basic configuration of the buoyancy pumping mechanism is shown in Figure 2. Here, pumping a sample to a chamber on the periphery of the disc induces the dissolution of a DF. This step prompts the release of the immiscible liquid, FC-40, which will settle underneath the sample liquid to displace it centripetally from a wide reservoir into a narrow channel. The pump rate is leveraged by the high difference between the cross sections of the outer reservoir and the narrow channel.

There are two major advantages of this approach. In the first case, the geometry of the large reservoir and the repeatable delivery of the pumping liquid FC-40 leads to a metering effect. The displacement by the FC-40 effectively splits the liquid into two discrete components, where the first, defined volume, is pumped radially inwards into the collection chamber while the other, overflow volume is pumped back the way it came in from. This effect can also be leverage to discretise samples for multi-parameter bio-assays [14].

The second advantage of this approach is its independence from the spin rate. The release of the FC-40 pumping liquid is only triggered when the aqueous sample wets and dissolves the DF. This independence from the spin rate effective makes the pumping module, an LUO, which can be placed between other steps without effecting upstream or down-stream processes.

We calculate the (equivalent) hydrostatic pressure:

$$p_\omega = \rho \Delta r \bar{r} \omega^2 \quad (1)$$

on a liquid element of density ρ, radial length Δr and mean radial position $\bar{r}$ rotating at the (angular) frequency ω. In the case of the centripetal pumping, the buoyancy force will be proportional to the density differences between the pumping and sample liquids. At a sufficiently large density differential and spin rates, the buoyancy force prevails over the capillary force which might counteract flow.

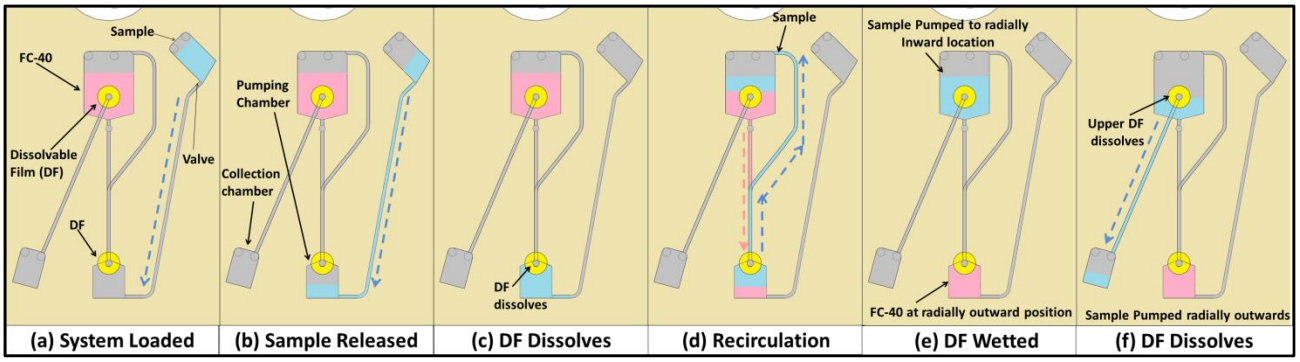

Figure 3: Schematic of reciprocating pumping (a) shows a disc cartridge in the initial configuration. The FC-40 is restrained by the lower DF (from entering pumping chamber) and upper DF (from entering the collection chamber). The sample is restrained behind a burst valve. (b) The spin rate is increased and the sample is pumped into the pumping chamber(c) the lower DF is wetted and the FC-40 is released. (d) FC-40 and sample establish a recirculating flow where the FC-40 displaces the sample upwards. This continues until equilibrium is reached. (e) The inner DF is contacted by aqueous sample and dissolves. (f) The sample is released to be subject to further LUOs.

4. CIRCULATION-DISPLACEMENT BASED PUMPING

In a further development of the buoyancy pumping mechanism, we introduce here displacement pumping through a side channel. In this approach, also sample is pumped into the inner reservoir initially holding the FC-40. Effectively, the addition of aqueous sample into the lower pumping chamber, which dissolves a DF film, establishes a circular fluid path where the denser FC-40 flows outwards through one channel and displaces the sample inwards through the other (Figs. 3 and 4). When the systems have reached equilibrium, the sample is layered above the FC-40 in the upper chamber. However, for practical use the microfluidic structure is configured such that, as the FC-40 flows radially outwards, another slow-dissolve DF is exposed to the aqueous sample and dissolves, thus allowing the sample to be subject of more LUOs.

The significant advantage of this method is it minimises the use of disc real-estate, particularly close to the centre of rotation. As the same reservoir is used both as the source of pumping liquid and as a destination for the sample, the critical footprint of this structure is effectively halved. A second advantage is the pumped sample, resting on top of the FC-40 as it is centrifugally pumped, generates an additional hydrostatic pressure and thus even slightly increases the speed of the pumping process.

5. BUOYANCY-BASED PUMPING TOWARDS INTEGRATED ASSAYS

Recently, we introduced a microfluidic structure that integrates buoyancy centripetal pumping with sample discretisation and metering [14]. To exemplify the applicability of this structure by a multi-parameter liver function bioassay panel integrated on a single disc [17]. Here an advancement is presented where a blood-processing chamber is placed an upstream and valved mixing chambers downstream. The integration of metering and discretisation of sample with centripetal pumping offers a number of advantages on the LoaD platform. In the first case, the capability of processing blood sample on the periphery of the disc, where a higher centrifugal field already emerges at smaller spin rates, lends the system to enhanced process integration. This, combined with the ability to move the discretized samples to a more central location and particularly useful in cases where multiple liquid handling steps are required and the available rotationally actuated valving technology restricts the spin rate envelope.

A second advantage is the combined discretizing / metering / pumping structure effectively acts as a valve. Before the heavy pumping liquid (FC-40) is released, the sample is effectively retained in the metering structure by the centrifugal force. Displaced upwards, it can be aliquoted into a number of discrete samples (Figs. 5 and 6 show 3-plex aliquoting) in a relatively small area and, through centripetal pumping, deliver them to distant locations. As the 'valve' the sample is pumped through is effectively an open channel it has a very small dead volume compared with some other valving technologies common on LoaD. A microchannel manufactured using the methods described here (cross-section: 0.5×0.086 mm^2) would, if it was extending 120 mm across the typical disc, have a maximum dead volume of ~5 µl. Additionally, unlike conventional DF valving technologies, these open channel 'valves' will be compatible with all non-aqueous liquids such as isopropyl alcohol (IPA) and ethanol (EtOH).

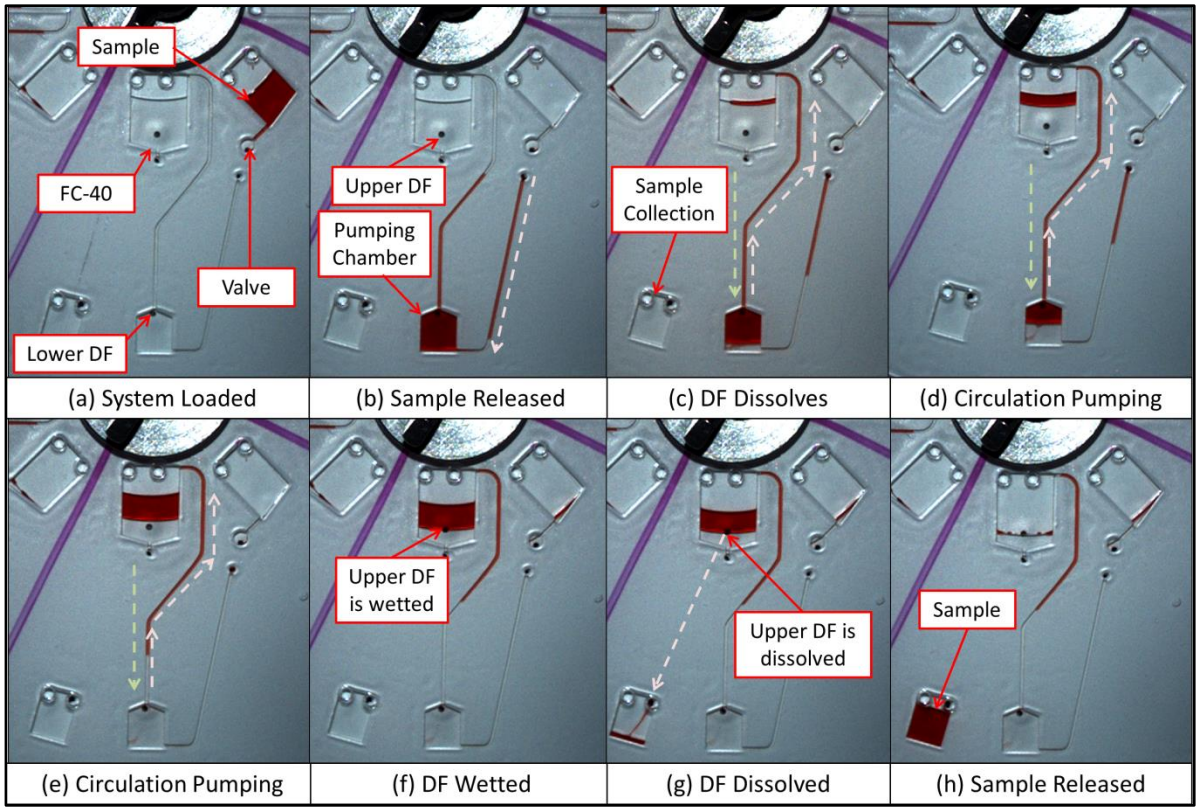

Figure 4: Video still acquired during circulation-displacement based pumping (a) shows a disc cartridge in the initial configuration. The FC-40 is restrained by the lower DF (from entering pumping chamber) and upper DF (from entering the collection chamber). The sample is restrained behind a DF burst valve. (b) the spin rate is increased and the sample is pumped into the pumping chamber (c) the lower DF is wetted and the FC-40 is released (d-e) the FC-40 and sample establish a recirculating flow where the FC-40 displaces the sample upwards. This continues until equilibrium is reached (f) the upper DF is wetted by aqueous sample and dissolves (g-h) the sample is released to be subject to further LUOs. Note that in the experiment above, the volumes of FC-40 was tailored to minimize both the volume of FC-40 passed to the next state in the test and the volume of sample lost during pumping. Varying (reducing) the volume of FC-40 from this optimal volume can permit the upper structure to also have a metering effect on the sample.

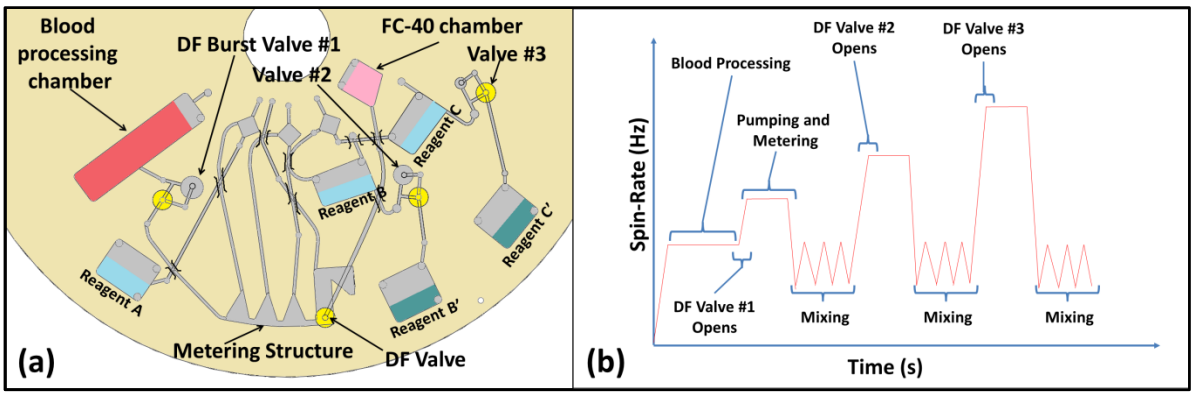

Figure 5: (a) Schematic of a disc used to demonstrate an integrated assay. This disc can demonstrate blood processing, discretizing, metering and pumping of extracted plasma and three stage delivery of reagents to selective chambers. In the planned bio-assay (i.e. liver function panel [17]) assays will run to completion or must be stopped after a specific time period through mixing sample with a 'Stop Reagent'. In the liquid handling protocol demonstrated, plasma mixed with Reagent A will run to completion (over ~15 minutes) while Reagent B reaction must be stopped after 3 minutes (through addition of Reagent B') while Reagent C reaction must be stopped after 15 minutes (Reagent C'). Absorbance measurements can then be made from read chambers on the edge of the disc. (b) Spin-protocol used to implement the assay. At spin rates below 25 Hz, blood may be stratified into cells and plasma. Increasing the spin-frequency to 30 Hz opens DF Valve #1. The plasma is then discretized, metered and pumped into Reagent A, B and C chambers. At this point, the reactions begin and the disc is rapidly accelerated and decelerated to enhance fluidic mixing. After a set time (3 minutes) the spin rate is increased to 45 Hz and the plasma / Reagent B solution is released to mix with the stop Reagent B'. Finally, 15 minutes after the pumping step, the spin rate is increased to 55 Hz to release the plasma / Reagent C solution to mix with Reagent C'. After a pre-defined mixing step the absorbance of each reaction can be measured.

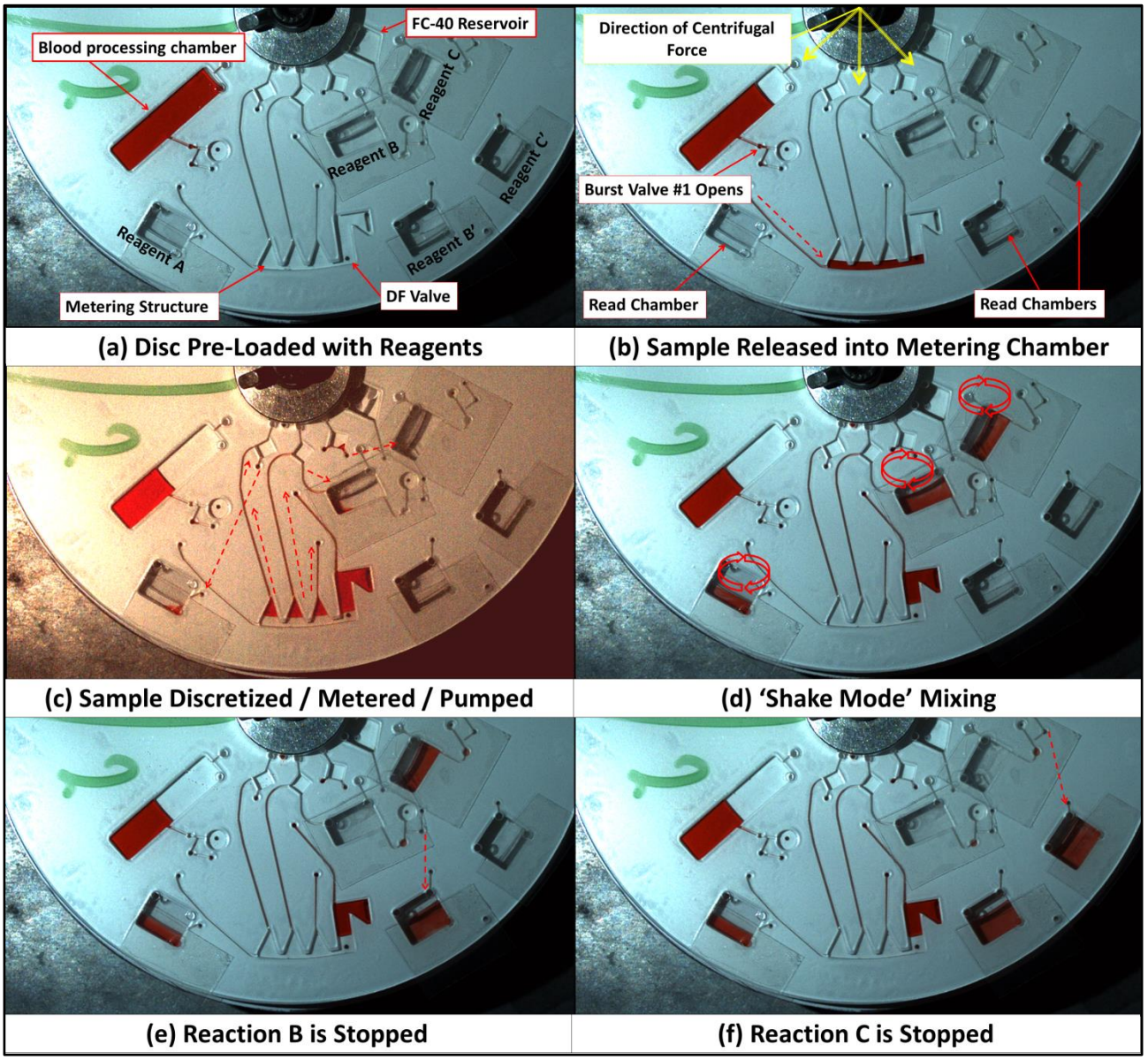

Figure 6: Disc designed for integrated bio-assays using integrated metering / mixing / pumping (a) shows the disc pre-loaded with FC-40 as pumping liquid, DI water to represent bio-reagents and dyed water to represent working sample (blood / plasma) (b) Increasing the spin rate to 30 Hz (see Fig 5) releases dyed water into the metering structure (c) the DF located in the metering structure dissolves and the FC-40 is released. Sample is discretized, accurately metered and pumped radially inwards. Previous measurements from a similar metering structure [14] indicate the accuracy of metering is 24 µl ± 240 nl (n = 9) (based on absorbance measurements of red food dye). (d) Sample is added to Reagent A, B and C and is mixed using the 'shake mode'-induced Euler force [20]. (e) After 3 minutes the spin rate is increased to 45 Hz and plasma / Reagent B solution is released into the read chamber where it mixes with the 'stop reagent' Reagent B'. (f) Similarly, after a further 12 minutes, the spin rate is increased to 55 Hz and the plasma / Reagent C solution is mixed with Reagent C'.

To demonstrate the capabilities of centripetal discretization / metering / pumping towards multi-parameter bio-assays we present a disc cartridge (Figs. 5 and 6) which implements all liquid handling steps associated with liver function monitoring [17]. Figure 5(a) shows a schematic of this disc design and Figure 5(b) shows a representation of the typical spin rate protocol to implement the following integrated assay:

1. Centrifuge Blood at 25 Hz – Stratify blood into RBCs and plasma
2. Increase spin rate to 30 Hz – Release plasma into metering chamber
3. Discretization / Metering / Pumping of 24 µl of plasma to chambers containing:
    a. 120 µl of Reagent A – an end-point absorbance measurement which runs to completion; similar to Albumin [17]
    b. 120 µl of Reagent B – an end-point absorbance measurement which must be 'stopped' prior to completion (after 3 minutes); similar to Total Bilirubin [17]
    c. 120 µl of Reagent C – an end-point absorbance measurement which must be 'stopped' prior to completion (after 15 minutes); similar to Alkaline Phosphotase [17]
4. Mixing of solutions
5. Transfer of plasma / Reagent B solution to a read chamber where it is mixed with a 'stop reagent' (Reagent B')
6. Mixing of solutions
7. Transfer of plasma / Reagent C solution to a read chamber where it is mixed with a 'stop reagent' (Reagent C')
8. Mixing of solutions
9. Reading of absorbance from read chambers.

Figure 6 shows video stills of the disc cartridge processing dyed water (in place of blood samples). With the disc spinning at 25 Hz (Fig. 6a) the 'blood' sample is held back by DF Burst Valve #1. Increasing the spin rate past the design burst frequency (to 30 Hz) results in the opening of this valve and the pumping of the dyed water into the metering structure. This structure fills rapidly (~30 s) (Fig. 6b) and, ~50 s after wetting the slow-dissolve DF, the pumping liquid (FC-40) is released. The FC-40 enters the chamber (Fig. 6c), and, as it displaces the liquid upwards, accurately meters and splits it into discrete fluid elements.

These volumes, representing plasma, are pumped radially inwards and then in the reverse direction towards mixing chambers. One chamber, pre-loaded with DI water representing Reagent A, is a read-chamber located on the periphery of the disc. The other, more central chambers are pre-loaded with DI water representing Reagent B and C initially sealed by DF valves which are designed to burst above 45 Hz and 55 Hz, respectively. With the sample delivered, the disc is accelerated and decelerated between 15 Hz and 22.5 Hz in order to induce 'shake-mode' style Euler mixing.

After a set time duration, the disc is accelerated to 40 Hz (Fig. 6e) and the food dye / DI water representing plasma / Reagent B is delivered to a read chamber (which has been pre-loaded with 60 µl of DI water representing 'stop' Reagent B'). Similarly, the disc is then accelerated to 55 Hz to process Reagent C (Fig. 6f).

6. CONCLUSIONS

In this work we have presented centripetal pumping techniques which offer a number of key advantages over existing methods. The use of a DF to restrain the pumping liquid FC-40 implies that the pumping mechanism is broadly independent of the spin rate and is only triggered with the presence of an aqueous liquid in the pumping chamber; this pumping technique is thus widely independent of upstream LUOs and thus useable in practice.

For circulation based pumping, by using a common chamber in which the FC-40 and sample effectively switch positions, the use of valuable, central real-estate is minimized. Additionally, the use of a DF valve in the upper chamber, which dissolves and opens a route for the sample as the filling level of FC-40 lowers, can act to meter the volume of liquid which is made available for subsequent LUOs. Furthermore, we have expanded our integrated discretizing / metering / mixing structure to demonstrate how it can become a key enabler of an integrated bio-assay. This structure demonstrates a number of decisive advantages including the ability to rapidly process blood; taking advantage of the elevated centrifugal field at the periphery of the disc and the ability to integrate this structure with time-triggered burst valves.

As future work, we intend to further optimize this structure and use it to implement the 3-plex multi-parameter liver panel described here.

ACKNOWLEDGEMENTS

This work was supported by Science Foundation Ireland (Grant No 10/CE/B1821) and the ERDF.

REFERENCES

[1] Madou, M. *et al.*, "Lab on a CD." *Annu. Rev. Biomed. Eng.* **8** (2006): 601-628.
[2] Ducrée, J. *et al.*, "The centrifugal microfluidic Bio-Disk platform." *Journal of Micromechanics and Microengineering* **17**.7 (2007): S103.
[3] Gorkin, R. *et al*., "Centrifugal microfluidics for biomedical applications." *Lab on a Chip* **10.**14 (2010): 1758-1773.
[4] Czugala, M., *et al.*, "CMAS: fully integrated portable centrifugal microfluidic analysis system for on-site colorimetric analysis." *RSC Advances* **3**.36 (2013): 15928-15938.
[5] Nwankire, C. E. *et al.*, "At-line bioprocess monitoring by immunoassay with rotationally controlled serial siphoning and integrated supercritical angle fluorescence optics." *Analytica Chimica Acta* **781** (2013): 54-62.
[6] Glynn, M. *et al.*, "Centrifugo-magnetophoretic Purification of CD4+ Cells from Whole Blood toward Future HIV/AIDS Point-of-Care Applications." *Journal of Laboratory Automation* **19**.3 (2013): **285-296**
[7] Hurst, J.W. *et al*., "Laboratory Robotics, a Guide to Planning, Programming and Applications" *VCH Publishers Inc.*, 1987.
[8] Kong, M.C. R. *et al.*, "Pneumatically pumping fluids radially inward on centrifugal microfluidic platforms in motion." *Analytical Chemistry* **82**.19 (2010): 8039-8041.
[9] Abi-Samra, K. *et al.*, "Thermo-pneumatic pumping in centrifugal microfluidic platforms." *Microfluidics and Nanofluidics* **11**.5 (2011): 643-652.
[10] Noroozi, Z. *et al*., "Electrolysis-induced pneumatic pressure for control of liquids in a centrifugal system." *Journal of The Electrochemical Society* 158.11 (2011): P130-P135.
[11] Zehnle , S. *et al.*, "Centrifugo-dynamic inward pumping of liquids on a centrifugal microfluidic platform." *Lab on a Chip*, **12** (2012): 5142-5145
[12] Kong, M.C.R. *et al.*, "Displacement pumping of liquids radially inward on centrifugal microfluidic platforms in motion." *Micromachines* **3**.1 (2011): 1-9.
[13] Soroori, S. *et al.*, "Design and implementation of fluidic micro-pulleys for flow control on centrifugal microfluidic platforms." *Microfluidics and Nanofluidics* **16**.6 (2013): 1117-1129.
[14] Kilcawley, N. A. *et al.*, "Buoyancy-Driven Centripetal Pumping for Nested Sample Preparation in Bioassays" *in Proceedings* of The 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2014), San Antonio, Texas, USA, October 26-30, 2014.
[15] Gorkin, R. *et al.*, "Centrifugo-pneumatic valving utilizing dissolvable films." *Lab on a Chip* **12**.16 (2012): 2894-2902.
[16] Kinahan, D. J. *et al.*, "Event-triggered logical flow control for comprehensive process integration of multi-step assays on centrifugal microfluidic platforms." *Lab on a Chip*, **14** (2014): 2249-2258
[17] Nwankire, C. E. *et al.*, "A portable centrifugal analyser for liver function screening." *Biosensors and Bioelectronics* **56** (2014): 352-358.
[18] Bartholomeusz, D. A. *et al.*, "Xurography: rapid prototyping of microstructures using a cutting plotter." *Journal of Microelectromechanical Systems*, **14**.6 (2005): 1364-1374.
[19] Grumann, M. *et al.*, "Visualization of flow patterning in high-speed centrifugal microfluidics." *Review of Scientific Instruments* **76**.2 (2005): 025101.
[20] Grumann, M. *et al.*, "Batch-mode mixing on centrifugal microfluidic platforms." *Lab on a Chip* **5**.5 (2005): 560-565.