**µFLU14-35**

# LABORATORY UNIT OPERATIONS ON CENTRIFUGAL LAB-ON-A-DISC CARTRIDGES USING DISSOLVABLE-FILM ENABLED FLOW CONTROL

**David J. Kinahan[1], Macdara T. Glynn[1], Niamh A. Kilcawley[1], Sinead M. Kearney[1], Jens Ducrée*[1],**

[1]Biomedical Diagnostics Institute; National Centre for Sensor Research; School of Physics; Dublin City University; Dublin; Rep. of Ireland

david.kinahan@dcu.ie, macdara.glynn@dcu.ie, niamh.kilcawley3@mail.dcu.ie, sinead.kearney24@mail.dcu.ie, jens.ducree@dcu.ie

## KEY WORDS

Microfluidics; Valving; Event-triggered; Sequential Actuation; Selective Routing

## ABSTRACT

*The suitability of the centrifugal "lab-on-a-disc" (LoaD) platform for point-of-use / point-of-care deployment, where ruggedness, portability, rapid turn-around times and ease-of-use are key, has resulted in increased interest from the academic community over the last decade. Recently, a new, so-called event-triggered valving paradigm was introduced which circumvented a number of the limitations of commonly used, rotationally actuated valves and complex instrument-controlled valving. In the dissolvable-film based event-triggered approach, it is the liquid movement about a disc which actuates a valve; thus enabling the concatenating of a number of liquid handling operations into an automated cascade. Functioning broadly independent of spin rate, the number of discrete valving operations is only limited by the available disc real-estate. In this work we present this valving paradigm to control a network of discrete Laboratory Unit Operations (LUOs) which, with experienced design, can be integrated together to implement complex fluidic assays. We describe how these valves can be configured, using Boolean-like network relationships, to implement LUOs such as sequential washing steps. We also describe how these valves can be configured to enable metering, mixing and selective routing of liquid flows. Finally, we describe how these valves can be configured to provide accurate temporal control of LUOs; thus providing an entire suite of process control technology which can be used to enable many bio-assays.*

## 1. INTRODUCTION

Increasingly over the past decade, the centrifugal microfluidic platform [1-2], or Lab-on-a-Disc (LoaD), has been applied to a variety of application fields such as biomedical diagnostics [3], bioprocess monitoring [4] and environmental screening [5-6]. The LoaD platform is particularly useful for near patient / point-of-care / point-of-use applications; deriving its advantages from the ease with which sample can be loaded and processed without need for pressurised fittings or external pumps. The cartridges typically have a form factor similar to commonly available optical discs (CD or DVD). Other major advantages of the LoaD platform are its compactness, the (often) basic instrumentation requirements (just a low-cost spindle motor), the inherent capability to centrifuge samples and its amenability to cost-efficient mass manufacture.

However, as all liquids on-disc are subjected to the same centrifugal field, valving has become a fundamental enabling technology which can enable on-disc mixing, metering, reagent release and other common Laboratory Unit Operations (LUOs) [7]. On-disc valving can be divided into three major categories; externally actuated, rotationally controlled, and event-triggered.

* Corresponding author

 

Externally actuated valves can broadly be defined as those where some periphery instrument interacts with the disc to open valves. These interactions can include provision of external pressure sources [5] for on-disc flow-control, thermal energy to induce phase-changes [8-12] or even physical manipulation [13]. These approaches greatly expand the capabilities of the centrifugal platform including expanding the number of LUOs on a single cartridge. However, the disadvantages include a more expensive, complex and often more fragile instrument, more complex / expensive cartridges and, in some cases, the need to stop the disc prior to valve actuation (which can compromise some sample preparation steps).

The more ubiquitous rotationally actuated valves use the variability of the centrifugal force, through changes in disc rotation speed, for actuation. Typically they are based around the interplay between centrifugally induced hydrostatic pressure and other forces acting on liquid elements such as pneumatic pressure or the capillary force. The high-pass version, which are actuate through increases in spin rate, include capillary burst valves [16-18], dissolvable film (DF) valves [19], burstable foils [20], elastomeric membranes [21] and dead-end pneumatic chambers [22]. Triggered by a reduction in spin rate, the low-pass variety includes both conventional siphons [4, 23-24] and pneumatically enhanced siphons [25-26].

The primary drawback of all rotationally-actuated is the fidelity of burst frequencies. Dependent on geometrically sensitive effects such as the capillary force, these valves are best described as having burst bands rather than burst frequencies. Sufficient leeway must be provided between the individual design burst frequencies to avoid overlap and valves actuating in the incorrect order. This requirement, combined with the necessarily finite range of spin rates available, limits the number of LUOs which can be placed in a sequential order. Combining low-pass and high-pass valves in series has mitigated this problem [4,23]; however, this strategy can be unreliable and also makes extensive use of disc real-estate.

The third valving class, event-triggered [27,28], is a recent innovation in which liquid movement about the disc triggers actuation of subsequent valving steps. Also called internally-triggered [27], this strategy uses the geometry of the disc to determine the order in which valves actuate, the movement of liquid to trigger the valves and can operate broadly independently of the spin rate. In one approach air-locks were created during disc loading which restrained the liquid due to a vacuum pneumatic pressure [27]. The movement of liquid on-disc opened a vent to these pneumatic chambers and thus released the restrained liquid. In another approach [28], based on dissolvable films (DFs) technology [19, 29-33], event-triggered valves can function akin to an electrical relay. Here, the valve is composed of a pneumatic chamber sealed by two films, called the Control Film (CF) and the Load Film (LF). Dissolving the CF vents the pneumatic chamber and permits the liquid to be released through the LF.

In this paper we present recent advances in this event-trigged DF valving technology [28]; describing them in terms of LUOs [7] such as metering, liquid routing and sequential actuation. We describe how these LUOs, which, enabled by the DF valves, now function independently of spin rate, and thus can be assembled in a modular fashion to enable complex on-disc liquid handling. Additionally, we describe how variations of the base event-triggered configuration, such as paper-triggered [29] and pulse-actuated valves [33], can provide increased temporal process control.

## 2. CARTRIDGE MANUFACTURE AND TESTING

### 2.1 Dissolvable Film Tabs

Dissolvable film is typically non-adhesive [19, 28] and so to incorporate it into discs they are mounted on double sided pressure sensitive adhesive (PSA). 86-µm thick double-sided PSA is acquired from the manufacturer (Adhesives Research, Limerick, Ireland) covered top and bottom by two protective carrier layers. The PSA is mounted on a standard paper cutter machine (CraftRobo Pro, Graphtec, USA) and the inner diameter of the DF tabs are cut from the upper protective layer and PSA. These PSA chads are then removed using a tweezers and the entire upper carrier layer is peeled away. The DF sheet is then rolled onto the exposed adhesive. A second cut is then used to define the tab outer diameters; cutting through the DF sheet and PSA layer. This results in the tabs mounted on the lower carrier layer and can be peeled off for use.

As previously described [28] two tab geometries are used. Typically, for the LFs, circular tabs are used which have a 1 mm inner diameter (ID) and 3 mm outer diameter (OD). For the CF tabs, a slot shape which has an inner area 1 mm wide, 4 mm long and a 1 mm thick PSA border is used. This shape allows the DF to be wetted without being entirely submerged; thus avoiding air-locking of pneumatic venting channels. Two grades of DF film are used. One film, KC 35 (Solublon®, Harke Packpro, Germany) takes ~40 s to dissolve in the presence of de-ionised (DI) water while the second grade, a low-cost film used for embroidery - called here E-film, (Barnyarns, Rippon, UK) - dissolves in ~6 s [28].

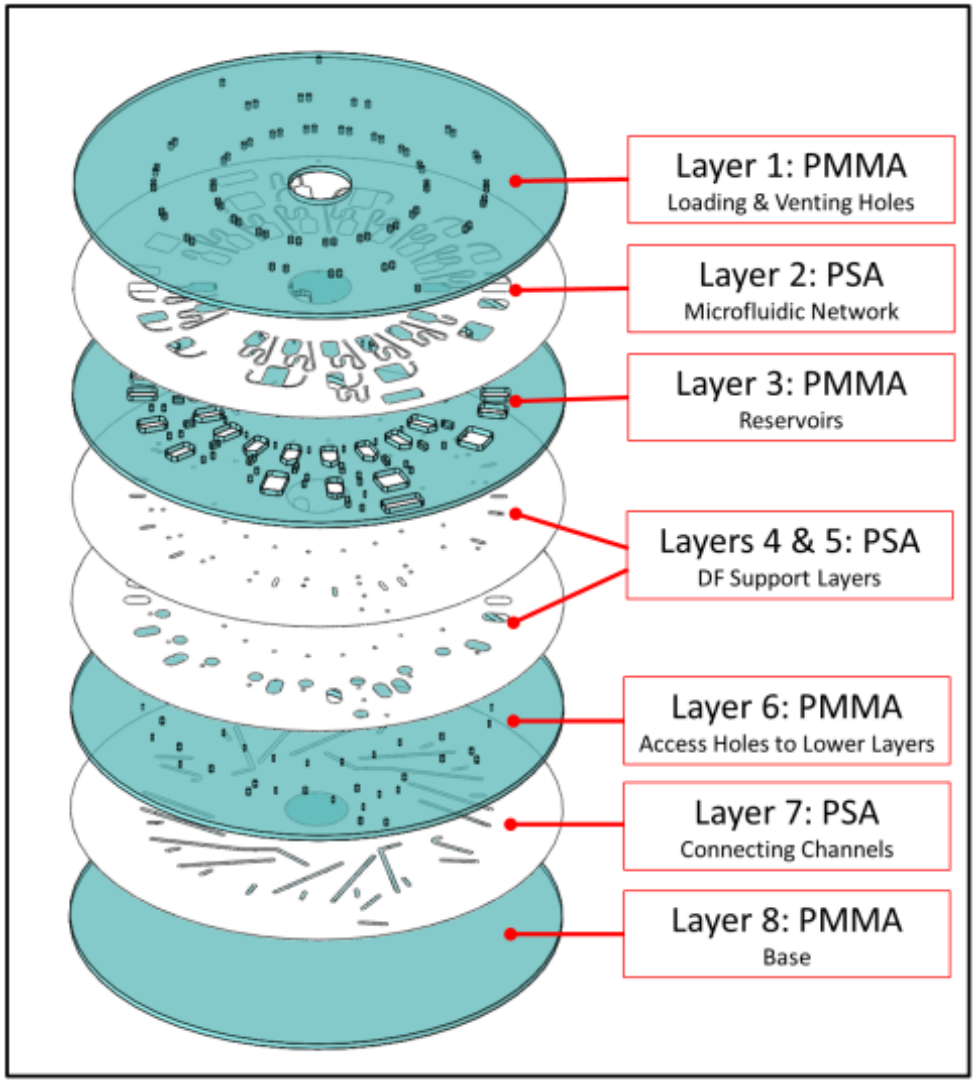


**Figure 1:** Exploded view of the disc cartridge showing the multi-layer architecture

### 2.2 Disc Assembly

The microfluidic discs were manufactured using multi-laminae (Figure 1) rapid prototyping techniques / xurography [34]. They were assembled on a custom alignment stand from layers of Poly(methyl methacrylate) (PMMA) and layers of PSA [19, 28, 34]. Microchannels were created from voids in PSA using a knife-cutter (the same material / equipment used to manufacture DF tabs). Voids were cut in PMMA layers (1.5 mm thick) using a laser cutter (Epilog Zing, USA) to create through-holes, reservoirs and vents. The discs are composed of 8 separate layers:

1. Vents (PMMA) – sample loading and pneumatic venting
2. Upper Microchannels (PSA) – pneumatic connections and liquid transport
3. Reservoirs (PMMA) – bulk liquid storage, metering, mixing, etc.
4. DF Cover (PSA) – adhesion and mechanical support of DF tabs
5. DF Support (PSA) – adhesion and mechanical support of DF tabs
6. Midlayer (PMMA) – mechanical support of DF tabs and connection to lower channels
7. Lower Channels (PSA) – pneumatic connection and liquid transport
8. Base (PMMA) – sealing and mechanical support

Note that the DF Support layer contains voids into which the DF tabs are placed and the DF Cover layer is then placed over this layer during assembly to ensure good sealing. Microchannels in the Upper Microchannel and Lower Microchannel layers are connected via vertical vias which extend through the Reservoirs and Midlayers. DF tabs placed in the DF support and DF Cover layers can therefore seal these vertical vias to create pneumatic chambers / valves. As the multi-level architecture isolates microchannels on Layer 2 and Layer 7 (and indeed on Layers 4 / 5 if required), channels can cross each other if required by the design.

### 2.3 Experimental Test Stand

Experimental images were acquired from a "spin stand" [35] described previously [28] Discs were spun on a motor (Faulhaber Minimotor SA, Switzerland). A stroboscopic light source (Drelloscop 3244, Drello, Germany) was synchronised with a sensitive, short-exposure time camera (Pixelfly, PCO, Germany) such that images are acquired from the disc at a set point during each rotation. Thus, a series of images are acquired where the disc appears stationary with well-defined features / channel walls; thus permitting liquid movement on disc to be observed.

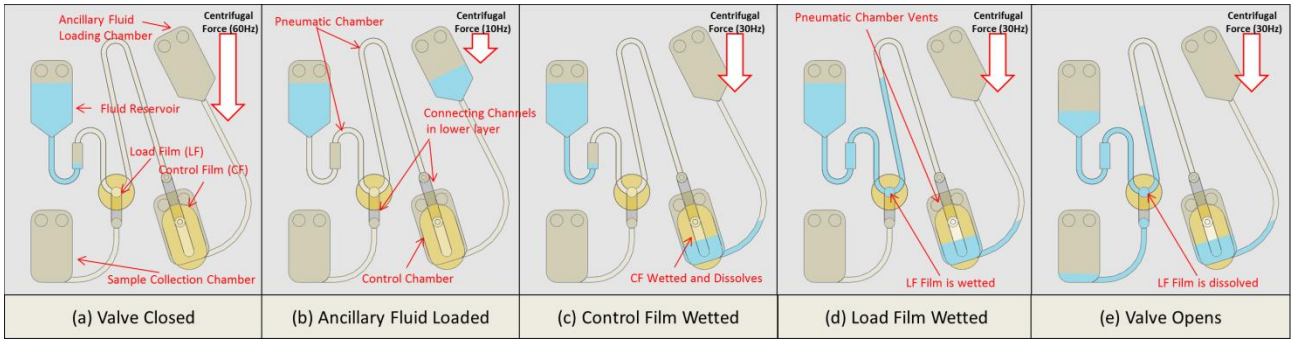


**Figure 2:** Schematic of the base event-triggered valve architecture. The valve is composed of a pneumatic chamber sealed by two DFs called the Load Film (LF) and Control Film (CF). (a) With both LF and CF sealed, the disc can be spin at high spin rates without the liquid reaching the LF (b) An ancillary, or triggering, liquid is loaded (c) Spinning the disc at a sufficient spin rate to pump the ancillary liquid results in the CF being wetted, dissolved and the pneumatic chamber venting (d) the sample can enter the valve and wet the LF. It is prevented from escaping through the CF orifice by a physical barrier (e) the LF dissolves and the liquid is released though the valve

## 3. BASIC VALVE FUNCTION

As previously described [28], the generalised form of the event triggered valve (Fig. 3) consists of a pneumatic chamber which is sealed by two DF films and the incoming liquid. In an analogy to an electrical relay the DFs are called the control film (CF) and the load film (LF). The pneumatic chamber is shaped so that, at spin rates typical of LoaD systems, the liquid cannot progress into the pneumatic chamber sufficiently to wet the LF. However, dissolving the CF by means of an ancillary liquid vents the pneumatic chamber. This permits the main liquid to advance to, wet, and then dissolve the LF. The main liquid now exits through the newly created opening.

The mechanics describing event-triggered valves are similar to those describing centrifugo-pneumatic DF valves [19,28]. The centrifugally induced hydrostatic pressure

$$p_\omega = \rho \Delta r \bar{r} \omega^2 + p_0 \qquad (1)$$

is calculated from the density of the liquid $\rho$, the radial length $\Delta r$ and the mean radial position $\bar{r}$ of the same liquid element as well as the (angular) rate of rotation $\omega$ and the ambient (atmospheric) pressure $p_0$.

Under increased centrifugal force, the sample will be displaced into the pneumatic chamber. This reduces the volume of the trapped gas within the chamber and increases its pressure. From Boyle's Law the pressure of the gas within the pneumatic chamber

$$p_c = p_0 \frac{1}{1 - \Delta V / V} \qquad (2)$$

depends on the total volume $V$ of the pneumatic chamber, and the reduction in gas volume due to the ingress of the liquid into the pneumatic chamber ($\Delta V$). These equations apply assuming the liquid is incompressible and interfacial tension is sufficiently strong to maintain a stable liquid-gas interface.

In order to ensure that, with the CF intact, the LF will not be wetted by liquid entering the valve at low spin rates, it is critical that the volume of the pneumatic chamber between the liquid entry and the LF, $\Delta V$, be sufficiently large relative to the entire pneumatic chamber, V. This is typically achieved using an upturned microchannel, where the denser liquid is radially outward the trapped gas, where an expansion locally increases the volume of the pneumatic chamber. A physical barrier, by means of the channel between the LF and CF extending radially inwards, prevents the sample escaping through the LF orifice. .

## 4. BOOLEAN ACTUATION

Along with the base configuration of the valves (Section 3), the valves can be extended to implement a number of different Boolean-like network relationships. These, in turn, enable the implementation of specific LUOs. In particular, the capability to sequentially release liquids using AND relationships is particularly powerful for 'sample-wash-elute' type bio-assays common in biomedical diagnostics.

In the simplest network relationship, the disc architecture can be configured such that liquid released from a first valve will wet the CF of a second valve, thus triggering a sequential cascade of valve actuation. This cascade release configuration can be further extended to provide logical flow-control elements. For example, as showing in Figure 3, an OR-relationship can be implemented though specifying a pneumatic chamber with one LF and two or more CFs. Thus the valve can be actuated by wetting any of these films.

Continuing the analogy of an electrical relay, AND-condition triggering can also be implemented. In this case, locating the CF of a valve so it can only be wetted by the aggregate liquid released from upstream valves will establish this condition. This is particularly powerful when used to release multiple valves which are draining into a common waste chamber. Similarly, locating two CF films where they will be wetted simultaneously will results in parallel triggering of two valves, releasing two liquid elements. This parallelisation can also be achieved through a configuration where multiple valves (multiple liquid elements each with a single LF) share a single pneumatic chamber, and thus can be triggered by dissolving a single CF. Finally, a configuration which is similar to DF burst valves [19], called co-located CF / LF valves, can also be implemented. In this approach, the valves, rather than bursting due to increased spin rate, are dissolved by an ancillary liquid. This results in the restrained sample liquid and ancillary liquid mixing; an important LUO at microfluidic length scales. Figure 4 shows a disc which uses 10 valves, all triggered at a constant spin rate, to demonstrate AND-trigger, parallel actuation of valves (using both mechanisms) and mixing using (co-)located valving. Note in particular that the time of valve actuation is, in this case, highly dependent on the speed at which the selected DF valves dissolve.

## 5. SELECTIVE FLUID ROUTING

Among the most powerful technique which can be implemented using these DF valves is selective liquid routing. Here, a co-located CF/LF valve is used to block one outlet of a bi-furcated channel (Figure 5). Due to air trapped between incoming liquid and this DF, the incoming liquid is routed down the other channel. Using an ancillary liquid to wet this DF 'from below' will dissolve the DF and thus open up the second channel for liquid flow. By ensuring the first channel has a larger hydraulic flow resistance, any subsequent incoming liquid will be preferentially routed down the second channel. A caveat, however, is the liquid routed down the second channel will mix with the ancillary liquid.

This method of selective routing is useful if the volume of ancillary liquid used can be kept to a minimum. It is particularly applicable to biomedical assays, such as silica-based nucleic acid extraction or Enzyme Linked Immunosorbent Assays (ELISA), which follow the 'sample > wash > elute' pattern as in these cases the eluate can be routed to a separate chamber for measurement or for further processing. Figure 5 illustrates a schematic showing how selective routing functions and Figure 6 shows a disc which has integrated metering, AND-triggering and selective routing; thus being potentially suitable for the aforementioned assays.

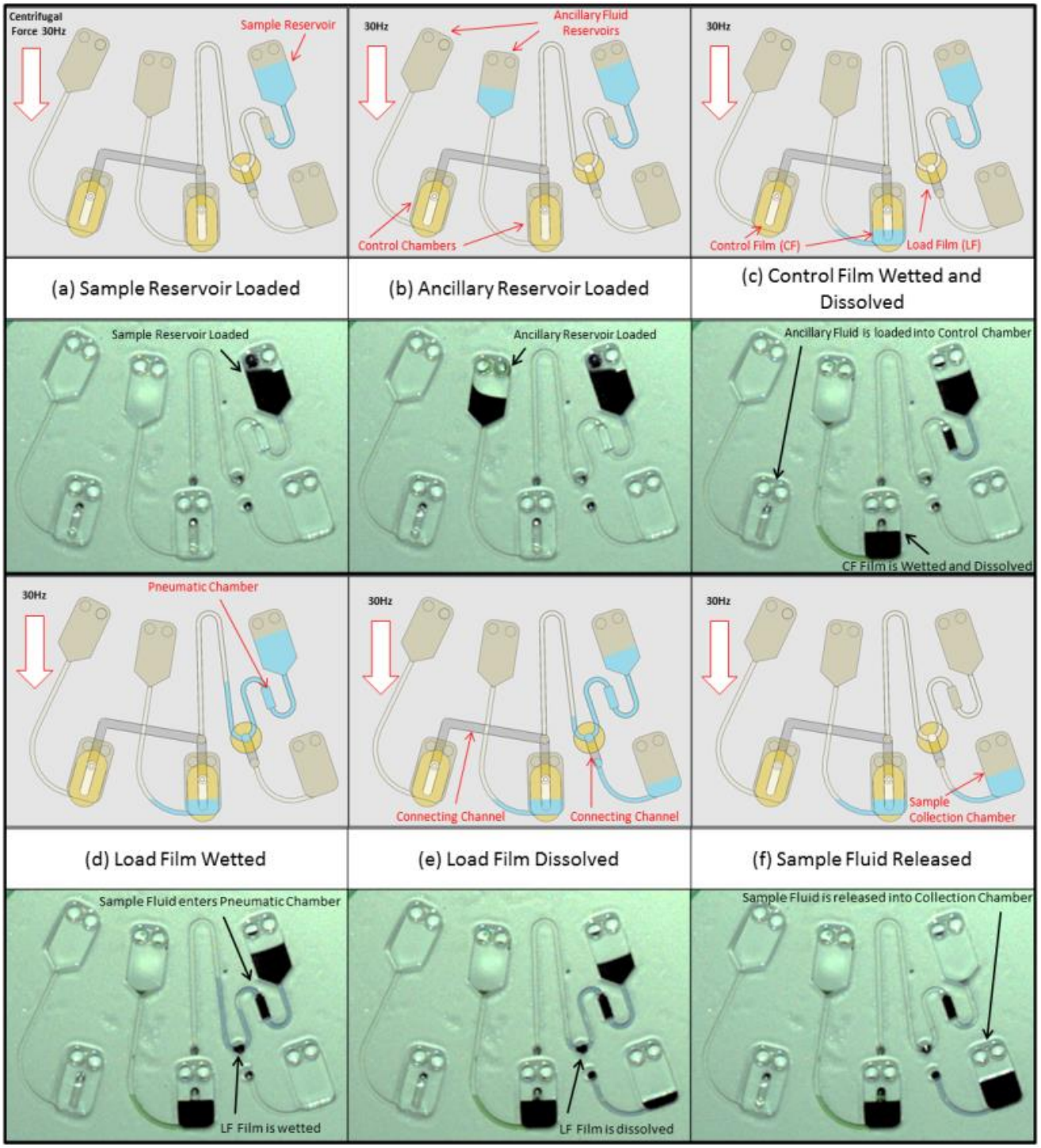


**Figure 3:** OR Boolean relationship where the addition of liquid to wet one or other CF will result in the release of liquid. In this case, the right-most CF is wetted (a) Sample is loaded and can hold at high spin rates (b) An ancillary liquid is loaded in the right-most ancillary liquid reservoir (c) the CF is wetted and the pneumatic chamber is vented. Note the chamber could equally be vented by dissolving the other CF (d) the LF is wetted (e) the LF is dissolved and the sample is released through the valve (f) the sample has fully flowed to the collection chamber.

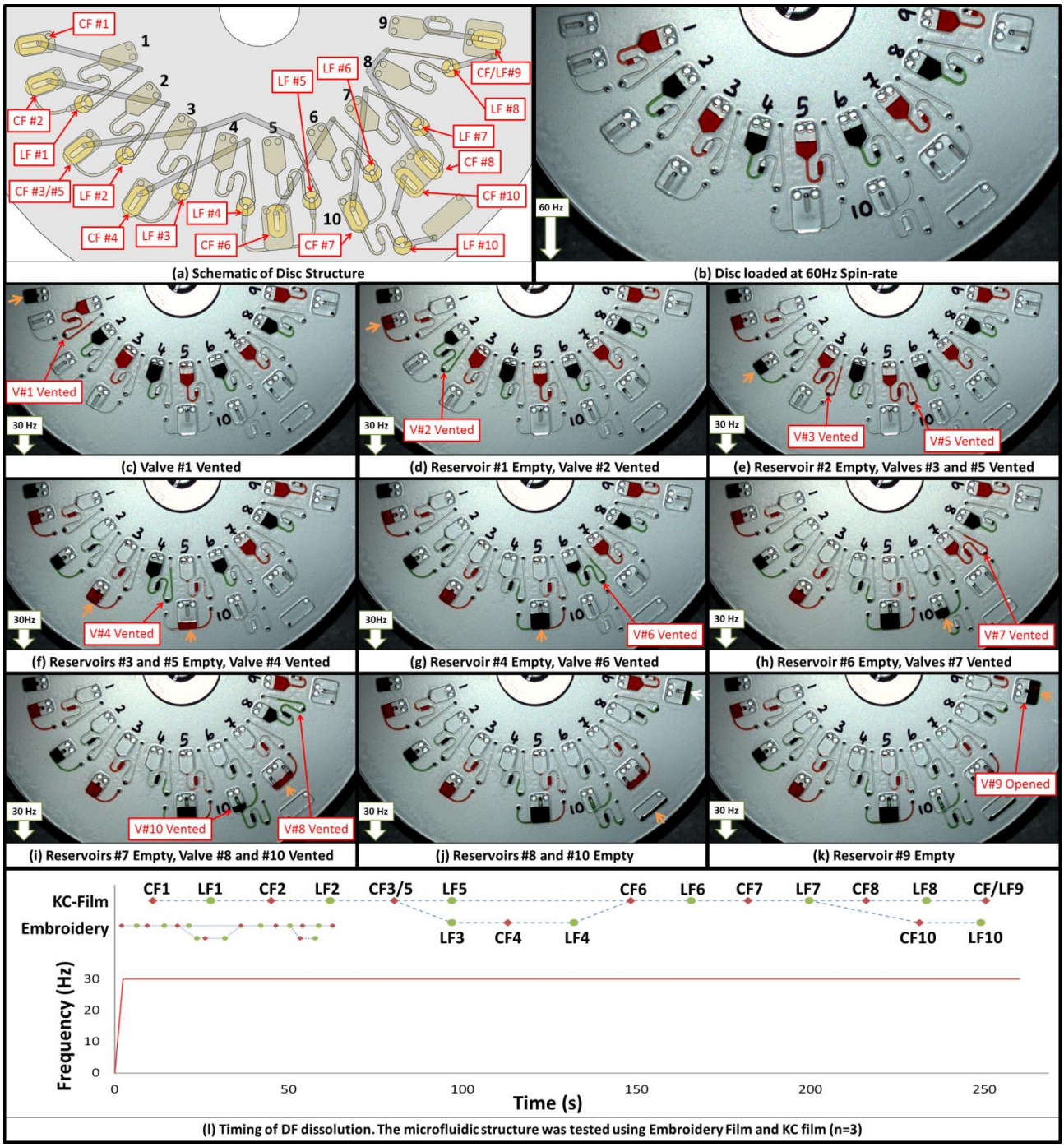


**Figure 4:** Fluid Network Cascade of 10 valves. (a) Schematic of the disc structure displaying the location of DFs and linking channels on the lower level of the disc structure. Note also that, for clarity, the components DFs of valves are labelled rather than the valves themselves (e.g. Valve #1 is constituted of CF#1 and LF#1). (b) Disc with all reservoirs loaded and the liquid subjected to high centrifugal field (60 Hz). (c-k) disc during the experimental sequence. In each figure, the liquid transfer (event) which triggered the valve actuation is highlighted using an orange arrow. Initially, ancillary liquid is added to the left-most reservoir to wet and dissolve CF#1; and thus initiating the cascade. The disc is rotated at 30 Hz for the duration of the test. Images shown are when the CF of each valve has dissolved and the pneumatic chamber has been vented. Note (e) shows parallel triggering of two valves (Valve#3 and #5) while (g) shows Valve#6 triggered using a logical AND relationship (Valves #4 and #5 must be opened) (i) also shows parallel triggering of Valves#8 and #10. (j-k) shows the liquid volume restrained by Valve #6 later restrained by Valve#10 and also demonstrates Valve#9 as a co-located valve. (l) time at which each film dissolved. The discs were manufactured and tested (n = 3) using two different grades of DFs. Opening of control films is shown by red diamond symbols while load films are shown by green circles. The blue dashed line shows the network dependencies between each valve. Note that time variation/error for each actuation step was less than ±1.2 s and for clarity error bars have been disregarded. As can be seen, discs manufactured using KC-film took over three times longer to complete than discs manufactured from the faster dissolving embroidery film. Reproduced from [28] by permission of The Royal Society of Chemistry (RSC).

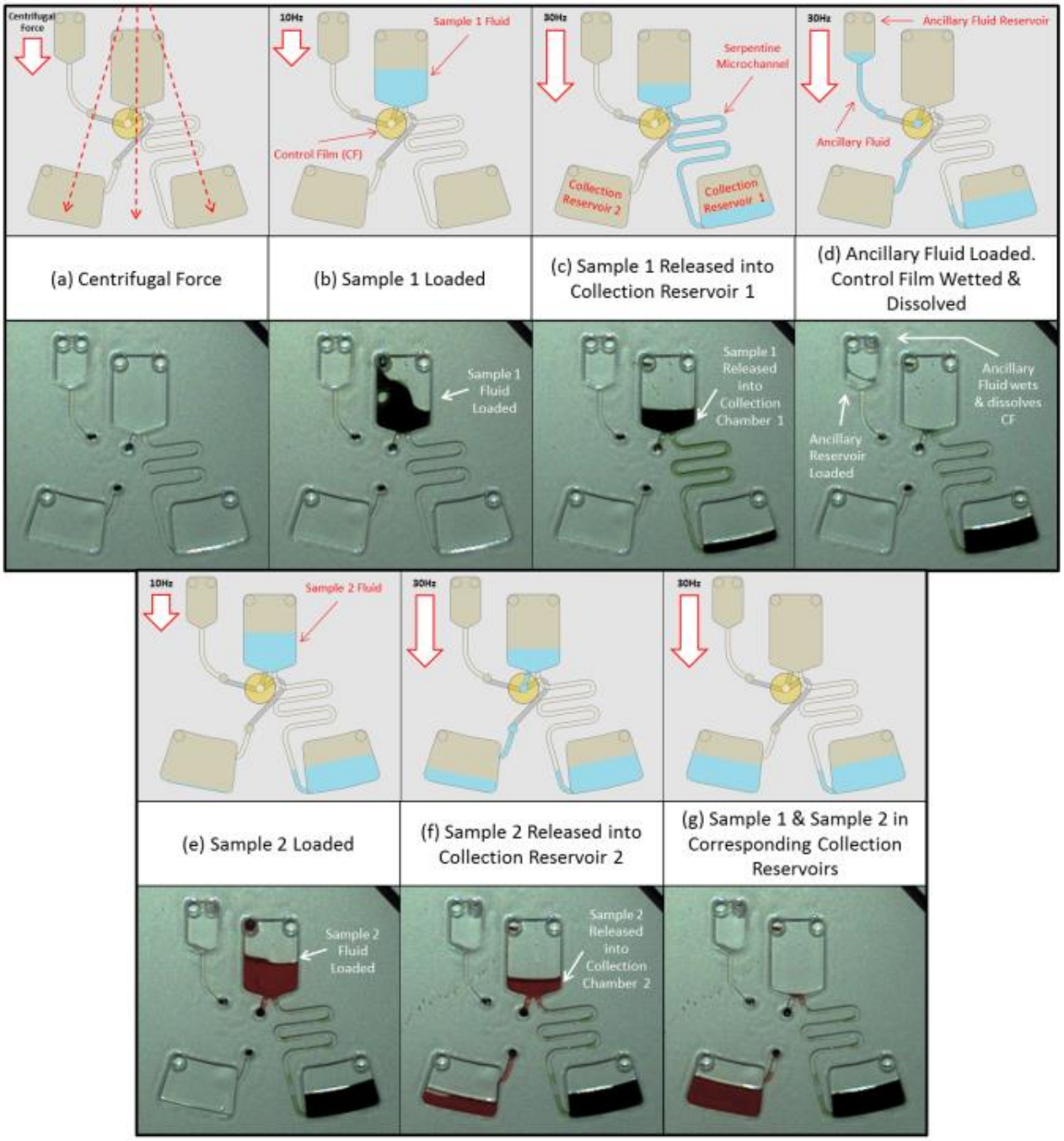


**Figure 5:** Co-located valving applied to selective fluid routing. (a) Basic structure. (b-c) Loading of sample and its routing past a DF film, recessed in a dead-end channel, and into collection reservoir 1. This can be repeated for multiple liquid elements. (d) Loading of ancillary liquid. This is routed past the DF and dissolves it 'from below', thus opening a route to collection reservoir 2. (e-g) Liquid added to the main reservoir is now routed to the collection reservoir 2.

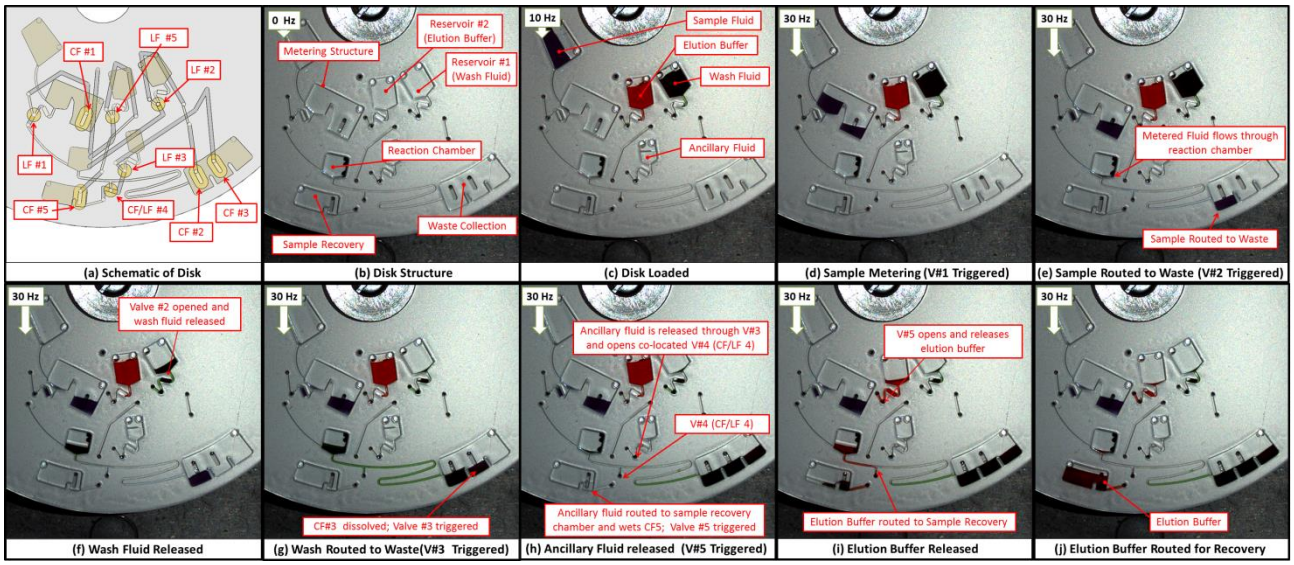


**Figure 6:** Metering and Routing. This disc architecture demonstrates how event triggered valves can be applied to a typical biomedical assay which utilises sequential liquid handling steps (such as silica-based nucleic acid extraction or ELISA). Here dyed water substitutes sample, wash buffer and elution buffer. (a) Schematic of the disc including linking channels on the lower levels of the design. Note, for the sake of clarity, only the CF and LF valves are labelled (e.g. Valve#1 (V#1) is composed of CF#1 and LF#1). (b) Shape of the structure. (c-j) Disc-based processing of the liquid. The experiment is initiated by accelerating the disc to 30 Hz and this spin rate is maintained throughout the experiment. The sample is metered and directed to waste through the reaction chamber. The wash buffer is then routed through the reaction chamber to the waste chamber. Finally, the elution buffer is guided through the reaction chamber to the sample recovery chamber. Note that in (d) the excess sample triggers the metering valve (V#1) and routing is achieved (h) by opening the co-located valve V#4 to open a passage for the sample to preferentially flow to the sample recovery chamber. While the preferential routing is based primarily on flow resistances, in some cases gas pockets trapped in downstream channels can increase the routing efficiency. Reproduced from [28] by permission of The Royal Society of Chemistry (RSC)

## 6. TEMPORAL CONTROL

The event-triggered valving technologies presented here represent a major advance on the state of the art. In particular, they function independently of spin rate and thus, unlike most rotationally actuated schemes, the number of sequential valving steps, and thus the number of sequential LUOs, is theoretically only limited by the available disc real-estate. In addition, the independence from spin rate allows the discs to operate on low-power, low complexity spindle motors.

However, as noted above the temporal control of the valves depends on the time taken for the DF to dissolve and the time for the liquid to travel around the disc. Depending on the available DF, this can result time spacing of order 120s per liquid handling step. However, this is far shorter than some incubations which may be required for some bio-assays. With this in mind, two solutions have been developed which can circumvent this limitation.

### 6.1 Paper Imbibition Based Actuation

In order to control the time taken for a liquid to reach a CF, cellulose paper can be used as a wicking media. Highly controllable, repeatable and low-cost, this media slows down the delivery of liquid to the CF and thus increases the time between valve actuations. Indeed, a particular advantage is a number of CFs can be placed along a single strip. In this approach, a single ancillary liquid element can wet the strip and, by wicking along it, trigger a number of steps sequentially. In this approach, the order of the valve actuation is defined by the position of the CFs on the strip and the timing is based on the rate of wicking and the space between the individual CFs. Figure 7 shows application of paper-imbibition to on-disc process control.

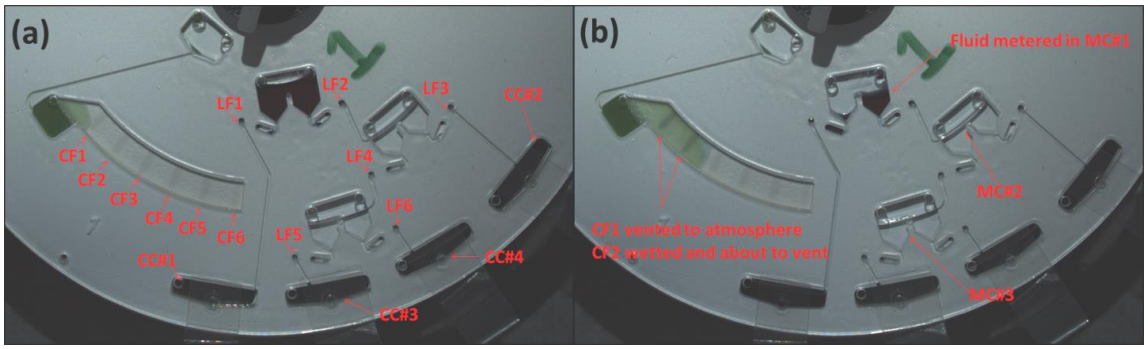


**Figure 7:** Valve actuation by paper imbibition. This disc is designed to implement a 4-fold serial dilution of a sample with the time available for mixing between steps governed by the progress of an ancillary liquid along a paper strip. As the actuations of the DFs are broadly independent of spin rate, the disc was subject to 'shake-mode' Euler mixing [36]. (a) Disc in its initial configuration just after reagents have been loaded and the paper-strip has been wetted by dyed water (green). (b) CF1 has been wetted and thus opened. This has removed food dye to Collection Chamber (CC) #1 and a metered volume remains in the Metering / Mixing Chamber (MC) #1. CF2 has wetted and will vent as soon as the DF loses its integrity. Figure reproduced from [29].

## 6.2 Pulse-based Actuation

In an alternative approach, pulse actuated valves combine the best features of DF burst valves with the best features of event-triggered valves. Here, the timing between individual valve actuations / LUOs can also governed by the spindle motor while the order of valve actuation is determined by the disc architecture. Effectively, the valve architecture is identical to a conventional event-triggered design except the LF is recessed in a dead end pocket. Here, with the CF sealed, the restrained sample, as in a conventional even-triggered valve, cannot reach the LF even at high spin rates. However, with the CF dissolved below a critical spin rate, the liquid can enter the valve but not wet the LF. Increasing the spin rate above a critical spin rate results, like a conventional burst valve, in the LF being wetted and the opening of the valve.

Similar to the even-triggered valves, these pulse-actuated valves can be placed in networks to enable functions such as sequential release, parallel release and AND-condition relationships. Assuming the spindle motor can decelerate below the critical burst frequency before the CF of a subsequent valve wetted, it is clear that a controlled pulse of the spindle motor will only open one valve in the series. With the CF of the subsequent valve dissolves, a second pulse with then open this valve and so on in sequence. Figure 8 shows a series of three pulse actuated valves where each valve is triggered at 120 s intervals by a 20 s pulse in spin rate.

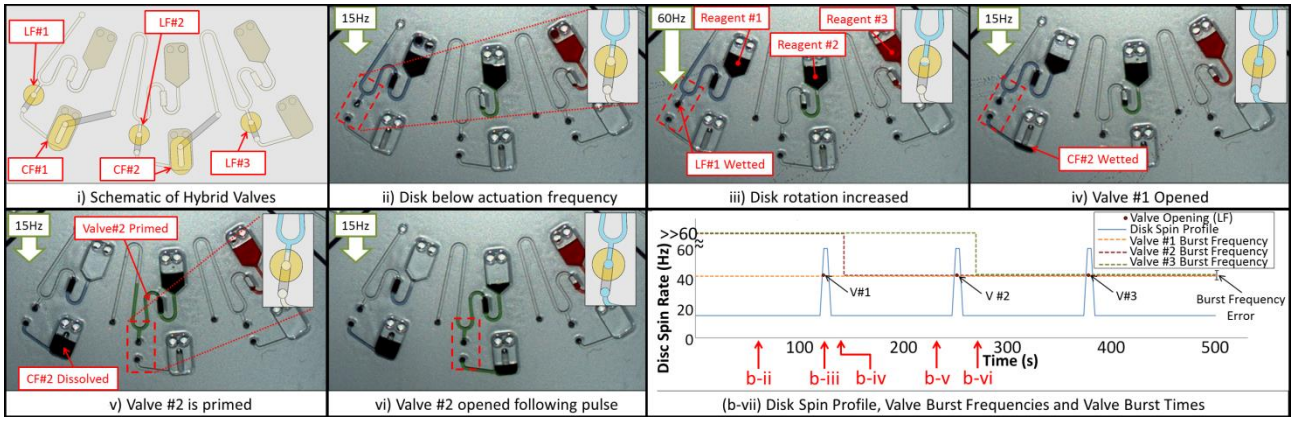


**Figure 8:** Pulse Actuated Valves. This cartridge illustrates the actuation of three valves in series at controlled time intervals. (i) Schematic of valve structure. Note the LFs are recessed into dead-end pockets (similar to DF burst valves [19]) (ii) below the critical burst frequency the valves do not open, (iii) increasing the spin rate results in the wetting of the first LF, (iv) the disc is decelerated before the LF dissolves and the subsequent CF is wetted, (v) the CF of the second valve is dissolved and the restrained sample enters the valve. However, it cannot wet LF2. (vi) A second pulse has wetted LF2, which now opens. (vii) Graph showing the relationship between spindle speed and burst frequency of each valve.

## 7. CONCLUSIONS

In this paper we have presented recent progress in DF-based valving scheme for LoaD systems. Particularly, we have shown how this technology enables LUOs such as liquid release (for washing and elution), metering, mixing and selective fluid routing. The event-triggered valves represent a new paradigm in on-disc valving technology; acting independently of spin rate they are highly reliable, enable use with low-cost spindle motors and permit the number of on-disc LUOs to be theoretically now limited only by available disc real-estate. We have also presented two alternative strategies which offer improved temporal process control on-disc.

## ACKNOWLEDGEMENTS

This work was supported by Enterprise Ireland under Grant No. CF/2011/1317, the ERDF, and the Science Foundation Ireland under Grant No 10/CE/B1821.

## REFERENCES

[1] Madou, M. *et al.* "Lab on a CD." *Annual 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] 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.
[5] Kong, M.C.R. *et al*., "Spectrophotometric Determination of Aqueous Sulfide on a Pneumatically Enhanced Centrifugal Microfluidic Platform" *Analytical Chemistry* **84**.22 (2012): 10038–10043
[6] Hwang, H *et al.*, "Lab-on-a-Disc for Simultaneous Determination of Nutrients in Water" *Analytical Chemistry* **85**. (2013): 2954-2960
[7] Hurst, J.W. *et al*., "Laboratory Robotics, a Guide to Planning, Programming and Applications" *VCH Publishers Inc*., 1987.
[8] P. Zucchelli, B *et al.*, Patent document WO200405024, 2004.
[9] Garcia-Cordero, J. L. *et al*., "Optically addressable single-use microfluidic valves by laser printer lithography" *Lab on a Chip*, , **10**.20 (2010): 2680-2687
[10] Lee, B. S. *et al*., "Fully integrated lab-on-a-disc for simultaneous analysis of biochemistry and immunoassay from whole blood." *Lab on a Chip* 11.1 (2011): 70-78.
[11] Abi-Samra, K. *et al*., "Infrared controlled waxes for liquid handling and storage on a CD-microfluidic platform." *Lab on a Chip* **11**.4 (2011): 723-726.
[12] Al-Faqheri, W. *et al.*, "Vacuum/compression valving (VCV) using parrafin-wax on a centrifugal microfluidic CD platform." *PloS one* **8**.3 (2013): e58523.
[13] Kawai, T. *et al*., "Rotatable Reagent Cartridge for High-Performance Microvalve System on a Centrifugal Microfluidic Device." *Analytical chemistry* **85**.14 (2013): 6587-6592.
[14] Chen, J. M. *et al*., "Analysis and experiment of capillary valves for microfluidics on a rotating disk." *Microfluidics and Nanofluidics* **4**.5 (2008): 427-437.
[15] Moore, J. L. *et al*., "Behavior of capillary valves in centrifugal microfluidic devices prepared by three-dimensional printing." *Microfluidics and Nanofluidics* **10**.4 (2011): 877-888.
[16] Thio, T. H. G. *et al*., "Theoretical development and critical analysis of burst frequency equations for passive valves on centrifugal microfluidic platforms." *Medical & Biological Engineering & Computing* **51**.5 (2013): 525-535.
[17] Li, T. *et al*., "Out-of-plane microvalves for whole blood separation on lab-on-a-CD." *Journal of Micromechanics and Microengineering* **20**.10 (2010): 105024.
[18] Haeberle, S. *et al*., "Centrifugal extraction of plasma from whole blood on a rotating disk." *Lab on a Chip* **6**.6 (2006): 776-781.
[19] Gorkin, R. *et al*., "Centrifugo-pneumatic valving utilizing dissolvable films." *Lab on a Chip* **12**.16 (2012): 2894-2902.
[20] van Oordt, T. *et al*., "Miniature stick-packaging–an industrial technology for pre-storage and release of reagents in lab-on-a-chip systems." *Lab on a Chip* **13**.15 (2013): 2888-2892.
[21] Hwang, H. *et al*., "Elastomeric membrane valves in a disc." *Lab on a Chip* **11**.8 (2011): 1434-1436.
[22] Mark, D. *et al*., "Aliquoting on the centrifugal microfluidic platform based on centrifugo-pneumatic valves." *Microfluidics and Nanofluidics* **10**.6 (2011): 1279-1288.

 

[23] Siegrist, J. *et al*., "Serial siphon valving for centrifugal microfluidic platforms." *Microfluidics and Nanofluidics* **9**.1 (2010): 55-63.

[24] Kitsara, M. *et al*., "Spin coating of hydrophilic polymeric films for enhanced centrifugal flow control by serial siphoning." *Microfluidics and Nanofluidics* **16**.4 (2014): 691-699.

[25] Gorkin, R. *et al*., "Pneumatic pumping in centrifugal microfluidic platforms." *Microfluidics and Nanofluidics* **9**.2-3 (2010): 541-549.

[26] Godino, N. *et al*., "Comprehensive integration of homogeneous bioassays via centrifugo-pneumatic cascading." *Lab on a Chip* **13**.4 (2013): 685-694.

[27] Ukita, Y. *et al.*, "Internally Triggered Multistep Flow Sequencers using Clepsydra." In Proceedings of the *16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2012), Okinawa, Japan, Oct*, pp. 1465-1467.

[28] 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

[29] Kinahan, D. J. *et al*., "Imbibition-Modulated Event-Triggering of Centrifugo-Pneumatic Cascading for Multi-Stage Dilution Series." *in Proceedings* of The 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2013), Freiburg, Germany, USA, October 27-31, 2014.

[30] Dimov, N. *et al,*. "Centrifugally automated solid-phase purification of RNA." In *Micro Electro Mechanical Systems (MEMS), 2014 IEEE 27th International Conference on*, pp. 260-263. IEEE, 2014.

[31] Nwankire, C. E. *et al,*. "Fluidic automation of nitrate and nitrite bioassays in whole blood by dissolvable-film based centrifugo-pneumatic actuation." *Sensors* **13**.9 (2013): 11336-11349.

[32] Nwankire, C. E. *et al,*. "A portable centrifugal analyser for liver function screening." *Biosensors and Bioelectronics* **56** (2014): 352-358.

[33] Kinahan, D. J. *et al.*, "Rotational-Pulse Actuated Dissolvable-Film Valves for Automated Purification of Total RNA from E. Coli " *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.

[34] Bartholomeusz, D.A. *et al.*, "Xurography: rapid prototyping of microstructures using a cutting plotter." *Journal of Microelectromechanical Systems* **14**.6 (2005): 1364-1374.

[35] Grumann, M. *et al.*, "Visualization of flow patterning in high-speed centrifugal microfluidics." *Review of scientific instruments* **76**.2 (2005): 025101.

[36] Grumann, M. *et al*., "Batch-mode mixing on centrifugal microfluidic platforms." *Lab on a Chip* **5**.5 (2005): 560-565.

 