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Centrifugally automated Solid-Phase Extraction of DNA by immiscible liquid valving and chemically powered centripetal pumping of peripherally stored reagents

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ABSTRACT

This paper presents two flow-control technologies for use on centrifugal Lab-on-a-Disc systems. The first, immiscible liquid valving, selectively blocks microfluidic channels using a high-density liquid fluorocarbon (FC-40). Used with a specific channel geometry, the FC-40 can permit liquid to enter a chamber but prevents it flowing back along the same path and so acts as "liquid" check-valve. The same liquid can be combined with a water-dissolvable film to provide an extremely robust liquid routing structure. The second technology uses CO_2 gas, created by wetting of commodity baking powder by water, to centripetally pump liquid from the periphery of the disc to the centre of the disc. The technologies are combined with valving schemes based on strategically placed, solvent-selective dissolvable films (DFs) to demonstrate repeated pumping of a liquid sample from the edge of the disc to the centre of the disc. The flow-control technologies are then combined to demonstrate fully automated Solid-Phase Extraction (SPE) of DNA with reagent storage on the periphery of the disc. We report an extraction efficiency of 47% measured relative to commercial spin-columns.

1. Introduction

The centrifugal microfluidic platform, or Lab-on-a-Disc (LoaD) (Madou et al., 2006; Ducrée et al., 2007; Gorkin et al., 2010a; Strohmeier et al., 2015; Tang et al., 2016; Smith et al., 2016; Miyazaki et al., 2020; Clime et al., 2019), has successfully demonstrated sample-to-answer automation of a comprehensive range of bioassays for a wide range of applications, including biomedical point-of-care diagnostics (Glynn et al., 2014), environmental monitoring (Kong and Salin, 2012; Hwang et al., 2013; Czugala et al., 2013) and quality control of industrial bioprocesses (Nwankire et al., 2013). The LoaD platform offers several benefits compared to conventional microfluidics. Centrifugal pumping from the centre of the disc to the periphery is enabled by a low-cost spindle motor rather than more specialised micro-pumps needed in most other Lab-on-a-Chip platforms. Typically, the LoaD possesses a similar geometry to common optical data storage media (e.g., CD™ or DVD™) and can be rotated by a simple spindle motor integrated on a rugged instrument of similar size to a commercial Discman™ (Czugala et al., 2013).

A major advantage of centrifugal microfluidics is the inherent ability to centrifuge during sample-preparation; this allows direct translation of many 'on bench' laboratory protocols to 'on disc'. This is particularly powerful for processing of blood including separation of plasma and isolation of white blood cells (WBCs) (Kinahan et al., 2014a, 2016a). With appropriate valving technology, the disc can automate other standard laboratory unit operations (LUOs) such as metering, mixing

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and aliquoting (Strohmeier et al., 2015). The LoaD can also be loaded directly by pipetting while open to atmosphere and so largely avoiding notorious 'world-to-chip' interfacing and priming issues. The LoaD cartridges are almost always self-contained and disposable leading to high levels of biosafety without the need to clean/decontaminate physical connectors. Similarly, low-cost spindle motors are ubiquitous and are cheaper than specialised pumps. These advantages provide a compact, highly autonomous, rugged, portable and affordable bio-analytical system which is particularly suitable for decentralised point-of-use testing.

A major challenge of LoaD systems is the centrifugal force only acts from the centre of the disc to the periphery. This naturally limits the number of sequential LUOs which can be placed in sequence. Thus, initial sample preparation steps such as blood separation (Torres Delgado et al., 2018) must be located centrally where the centrifugal force is lowest. Similarly, liquid reagents required for washing substrates or mixing with samples must also be located near the centre of the disc where 'real estate' is most scarce. For example, in a CD-format disc, with an outer diameter of 12 cm and and inner hole measuring 1.5 cm, more than 30% of its surface area is located within the 1 cm ring at the outer edge while less than 8.5% is contained within the innermost ring of the same width. To circumvent the small amount of central real-estate several options have been explored. Often, users will deviate from the standard CD™ or DVD™ formats by creating discs, or segments thereof, which have smaller of even no central holes, larger outer diameters, or simply thicker is standard to permit more reagent be stored near the centre of rotation (Kinahan et al., 2015a). Unfortunately, a drawback of this approach is it may preclude leveraging infrastructure and expertise from established optical disc manufacturers. Alternatively, various centripetal pumping technologies have also been developed and these are described in Table 1.

Chemical energy has also been used for displacement pumping. Recently, oxygen was released from on-disc stored hydrogen peroxide for bubble based mixing (Burger et al., 2016); a similar approach was used to enable radially inwards pumping (Krauss et al., 2018) using CO_2 released from the reaction of sodium bicarbonate and sulfuric acid. Chemically powered pneumatic displacement pumping in conventional (non-centrifugal) microfluidic chips has been demonstrated (Ahn et al., 2004; Weigl et al., 2008), including the liquid-induced activation of commodity baking powder (Fordtran et al., 1984).

The automation of molecular diagnostic (nucleic acid) assays on Lab-on-a-Disc systems has become increasingly common (Van Nguyen et al., 2019a) due to its potential for application in a wide range of areas from medical diagnostics to food safety testing at point-of-care/point-of-use. SPE (Park et al., 2014, 2017; Kinahan et al., 2016b; Liu et al., 2018; Oh et al., 2016; Stumpf et al., 2016; Van Nguyen et al., 2019b; Jung et al., 2015) is often a key sample preparation step to remove inhibitors from DNA or RNA samples prior to nucleic acid amplification (i.e. PCR (Stumpf et al., 2016; Focke et al., 2010) LAMP (Phaneuf et al., 2018; Loo et al., 2019; Cao et al., 2018), RPA (Chen et al., 2018; Choi et al., 2016; Schuler et al., 2015), and NASBA (Brennan et al., 2017)).

In this work, we first demonstrate the use baking powder as a store of CO_2 for displacement pumping of liquids radially inwards. We do this by combining event-triggered dissolvable film valving, which controls the wetting of baking powder and hence the release of CO_2, with novel liquid check-valves which prevents liquid being pumped backwards through the microfluidic structure. These technologies are then used to demonstrate reciprocal pumping of a liquid sample from the centre to the periphery of the disc three times with minimal loss. Next, we demonstrate that the check-valves can enable centrifugo-pneumatic centripetal pumping based only on changes in disc spin speed (i.e. without any baking powder present). For this demonstration, we use an identical microfluidic structure but with baking powder and dissolvable films removed.

Finally, we utilise the basic CO_2 powered pumping concept, combined with rotationally-actuated DF valves, to demonstrate reagent storage on the periphery of a disc for automating a common nucleic acid purification protocol delivering an efficiency of 29% relative to the initial DNA concentration or 47% relative to the performance of a 'gold standard' commercially available spin columns.

Table 1
Description of centripetal pumping technologies.

Type/Description	Advantages	Disadvantages	Reference
External Compressed Air from Cylinders	Simple technology	Requires additional instrumentation. Not reliably controlled	Kong and Salin (2010)
Integration of micro-air pumps into spindle/ pneumatic slip-ring with pneumatic valves.	Flexible and enabled a wide range of LUOs to be implemented.	Requires additional instrumentation.	Clime et al. (2015)
Thermo-pneumatic pumping. Air is locally heating on the Lab-on-a-Disc	Simple technology.	Requires additional instrumentation.	Abi-Samra et al. (2011)
Electrolysis of liquids generates gas to displace reagents.	Simple technology	Requires additional instrumentation.	Noroozi et al. (2011)
Rotationally stored energy. Energy is trapped in a dead-end pneumatic chamber during disc acceleration (gas compression) and released during deceleration.	Simple technology to implement. Requires just disc spindle-motor. Liquid can be routed using inlet and outlet channels of differing hydraulic resistance. Can enable metering and aliquoting.	May require powerful spindle motor to provide high rates of acceleration and deceleration.	(Zehnle et al., 2012; Schwemmer et al., 2015; Gorkin et al., 2010b)
Displacement pumping (air or immiscible liquid)	Requires just a spindle motor.	Can require complex loading procedures or use of valves on-disc to enable operations.	(Kong et al., 2011; Soroori et al., 2014; Miyazaki et al., 2018)

2. Materials and methods

2.1. Disc manufacture and assembly

The microfluidic cartridges used in this study were assembled using multi-lamination methods from four layers of Poly(methyl methacrylate) (PMMA) and four layers of PSA (Pressure Sensitive Adhesive, Adhesives Research, Limerick, Ireland) (Kinahan et al., 2014b). Xurography (Bartholomeusz et al., 2005; Kinahan et al., 2016c) (using a commercial knife-cutter from Graphtec, Yokohama, Japan) was used to create voids in the PSA layers (86 μm thick) which resulted in microchannels and other small features. Larger structures, such as reservoirs for reagent storage, baking powder storage and sample collection, were patterned in PMMA layers (1.5 mm thick) using a laser cutter (Epilog Zing, USA). These layers were aligned on a custom assembly jig. Between each alignment step, the attachment of layers was reinforced using a hot-roll laminator (Hot Roll Laminator, Chemsultant Int., USA). Each DF was mounted on PSA tab using previously described methods (Kinahan et al., 2014b, 2016c) and then manually positioned within the disc during manufacture.

2.2. Experimental test stand

All discs in this study were imaged using an experimental test platform commonly referred to as a "spin stand" (Grumann et al., 2005). The majority of discs were tested using a computer-controlled motor (Faulhaber Minimotor SA, Switzerland). An encoder embedded on the spindle provides two signals (1 pulse/rotation and 1000 pulses/rotation); custom hardware sets the angular position where a digital trigger activates a stroboscopic light source (Drelloscop 3244, Drello, Germany) and a sensitive, short-exposure time camera (Pixelfly, PCO, Germany). Some discs were tested on an alternative spin stand which uses same model stroboscopic light source. However, on this system, the spindle motor (Festo EMME-AS-55-M-LS-TS, Esslingen, Germany) has a built-in encoder which generates a pulse at a programmatically controlled shaft-angle and is controlled with a custom LabVIEW program via a CANOpen interface. A Basler Ace 2040-90uc (Basler, Germany) camera was used for stroboscopic imaging. All discs are tested at a rotational frequency of 30 Hz except for the pneumatically driven pumping disc (described in Section 3.3.1 and Movie 3, ESI) and the DNA purification disc (spin protocol shown in Fig. 7b). Acceleration/deceleration ramps were 12.5 Hz s^{-1}, except the pneumatic-pumping disc where deceleration was at 2 Hz s^{-1}.

2.3. DNA purification and quantification

To demonstrate the capability of this centripetal pumping architecture to purify DNA, we use DNA standards, provided as part of the Quant-iT™ PicoGreen® dsDNA (ThermoFisher), as a pseudo-sample. This standard is well defined and used to calibrate the quantification kit. The DNA purification disc uses reagents adapted from QIAquick PCR Purification kit (Qiagen, Hilden, Germany); the extracted DNA in the eluate and was quantified using a Quant-iT™ PicoGreen® dsDNA (ThermoFisher). First, the DNA standard provided with the PicoGreen kit was diluted in TE Buffer to a concentration of 2 μg mL^{-1}. A working solution of Quant-iT™ PicoGreen® reagent is also prepared as per supplier's protocol. For processing in the conventional spin column and on disc, the 2 μg mL^{-1} is diluted 5:1 using buffer PB (provided in the QIAquick kit).

For both purification methods, a sample of 60 μl is loaded. For the QIAquick columns, this sample is then washed using 750 μl of PE Buffer

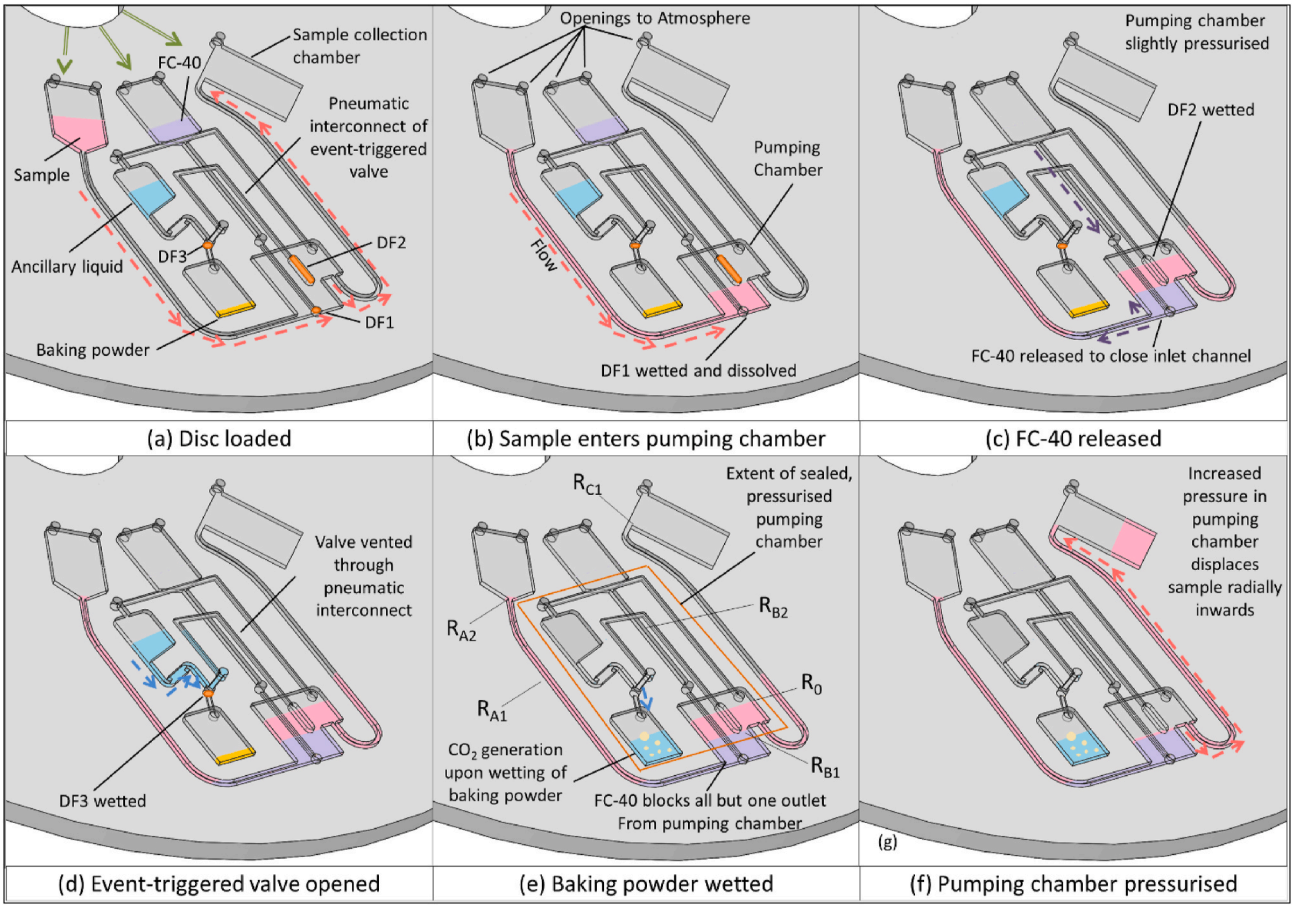

Fig. 1. Centripetal pumping structure. Note in the multilayer disc shown in Fig. 2 some connecting channels are hidden as opaque materials are used during manufacture. (a) On-disc stored reagents. The green arrows indicate the direction of centrifugal force and the dashed red line shows the nominal path of the sample. DF1 restrains FC-40, which acts as a valving liquid, while DF2 and DF3 are constituent parts of an event-triggered valve (b) Upon spinning the disc, the sample is centrifugally driven into the pumping chamber. DF1 is wetted and thus dissolves to release the FC-40 valving liquid. Note that the loading chamber is open to atmosphere and the pumping mechanism is triggered by the presence of the sample. Thus, once spinning quickly enough to pump the sample liquid into the pumping chamber, the centripetal pumping mechanism is broadly independent of the specific spin protocol. (c) High-density FC-40 is released to close the inlet channel by settling under the sample liquid. This valving liquid also slightly displaces the sample liquid further into the pumping chamber. DF2 is wetted and begins to dissolve. (d) With DF2 dissolved, the event-triggered valve is vented so the ancillary liquid can wet and dissolve DF3 (e) The release of the ancillary liquid (DI water) through the DF3 orifice to wet the baking powder fuels the generation of CO_2. Note that the solid orange rectangle represents the extent of the pressurised (pumping) chamber. (f) As the high-density FC-40 effectively seals the inlet port, the gas release preferentially displaces the sample liquid radially inwards to the sample collection chamber. A prior version of this figure was published in (Kinahan et al., 2015b) and is adapted here with kind permission of IEEE. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and Ethanol (EtOH) based buffer provided in the QIAquick kit; on disc, two 100 μl washes are loaded into the chambers called EtOH #1 and EtOH #2. For both, the QIAquick columns and on disc, the elution step is performed using 60 μl of EB buffer (again provided in the QIAquick kit). For quantification, 30 μl of the sample, as recovered from the QIAquick column, from the disc and from the reference DNA (2 μg mL^{-1}), are each added to 30 μl of the working solution of Quant-iT™ PicoGreen®. Fluorescence is measured using a commercial plate reader (Tecan) per the Quant-iT protocol and, for reporting, fluorescent signal is normalised relative to the reference (2 μg mL^{-1} DNA) fluorescent signal.

3. Pumping and valving – concepts and application

3.1. Reciprocal pumping

The pumping system is based upon pressurising a pneumatic chamber through release of CO_2 gas from wetted baking powder and so displacing the liquid radially inwards (Figs. 1–5). Reciprocal pumping of a sample is described in Figs. 1 and 2 (see also ESI Video 1 and 2). Here, the disc is rotated at 30 Hz and a sample (coloured water) is pumped to a chamber on the outside of the disc. This chamber has three fluidic connections: inlet, the sample exit, and a channel, blocked by a dissolvable film, which retains FC-40 (Sigma Aldrich, USA). FC-40 is a high-density (specific gravity of ~ 1.85) inert fluorocarbon which is immiscible in aqueous which does not dissolve the DFs used in this study. FC-40 is widely used in microfluidics and has shown to be compatible with a wide range of bio-assays such as cell-culture (Soitu et al., 2018) and PCR (Walsh et al., 2006). Additionally, recessed within the chamber, is a reservoir of water which is kept from a store of baking powder by an event-triggered DF valve (Kinahan et al., 2014b).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.biosx.2021.100085

When the sample enters the chamber, the DF initially retaining the FC-40 is wetted and dissolves to release the FC-40. This immiscible ancillary liquid then displaces the sample radially inwards to block the inlet channel, while the sample closes the outlet channel to create a sealed gas pocket. Next, an event-triggered valve is opened to wet the baking powder; the subsequent release of CO_2 increases the pressure in the enclosed gas pocket. To balance this increased pressure, the FC-40 is displaced up both the inlet and FC-40 arm, while the sample is displaced to the outlet channel. However, due to the density difference between FC-40 and the sample (water), the sample is pumped inwards to a collection chamber while the FC-40 remains in the outer chamber.

Fig. 3 shows a typical configuration of this system as sample is about to be pumped radially inwards. In the pneumatic chamber, the pressure increase in the chamber is based upon displacement of liquid into the chamber (p_{comp}) and the release of the CO_2 from baking powder (p_{BP}).

Based upon Boyle's law, we can define the gauge pressure due to centrifugal compression, $p_{\Delta V}$, as:

$$p_{\Delta V} = \frac{p_0 V_0}{(V_0 - \Delta V)} - p_0 \tag{1}$$

where p_0 is atmospheric pressure (pressure in the chamber before it is sealed), V_0 is the total volume of the chamber which can be sealed, and ΔV is the volume reduction of the trapped gas due to the displacement of liquid into V_0.

Neglecting any dynamic effects, it holds that the centrifugally generated hydrostatic pressure in any of the microchannels arms (inlet arm (ArmA), FC-40 arm (ArmB) or outlet arm (ArmC)) is equal to the gas pressure in the pumping chamber when measured at the gas-liquid interface (datum point R_0 shown in Fig. 3).

$$p_{ArmA} = p_{ArmB} = p_{ArmC} = p_{\Delta V} + p_{BP} \tag{2}$$

It must be noted that, on a rotating structure, the centrifugally generated hydrostatic pressure (which is analogous to hydrostatic pressure $P = \rho g h$ for water of density, ρ, and subject to gravity, g, when measured at a depth of h from the surface) is given by the equation (Madou et al., 2006):

$$\Delta P = 0.5\rho\omega^2\left(R_2^2 - R_1^2\right) = \rho\omega^2\bar{r}\Delta r \tag{3}$$

where ρ is the density of the liquid, ω is the angular spin rate, and R_1 is the radially inward location of the fluid element and R_2 is the radially outwards location of the fluid element across which the pressure difference, ΔP, is measured. In alternative but mathematically equivalent notation commonly used, the radial distance between the two measurement points $\Delta r = R_2 - R_1$, and the average radial position between these two points, $\bar{r} = (R_2 + R_1)/2$, is used.

Referring to Fig. 3, the inlet channel (ArmA) is plugged with FC-40 and a small component of sample. ArmB, the FC-40 valve, is typically filled entirely with FC-40 while the outlet channel ArmC is filled entirely with the aqueous sample:

$$p_{ArmA} = 0.5\omega^2\left[\rho_w\left(R_{A1}^2 - R_{A2}^2\right) + \rho_{FC}\left(R_0^2 - R_{A1}^2\right)\right] \tag{4}$$

$$p_{ArmB} = 0.5\omega^2\left[\rho_{FC}\left(R_0^2 - R_{B2}^2\right)\right] \tag{5}$$

$$p_{ArmC} = 0.5\omega^2\left[\rho_w\left(R_0^2 - R_{C1}^2\right)\right] \tag{6}$$

where the angular spin rate is ω and the densities and of water and FC-40, respectively, are ρ_{FC} and $\rho_{w,}$.

As the ρ_{FC} significantly exceeds $\rho_{w,}$ the aqueous sample is shifted radially inwards as the system seeks equilibrium. The sample is pumped radially inwards through ArmC and, as it enters the sample collection chamber, at R_{C1}, the liquid column becomes discontinuous. Thus, the

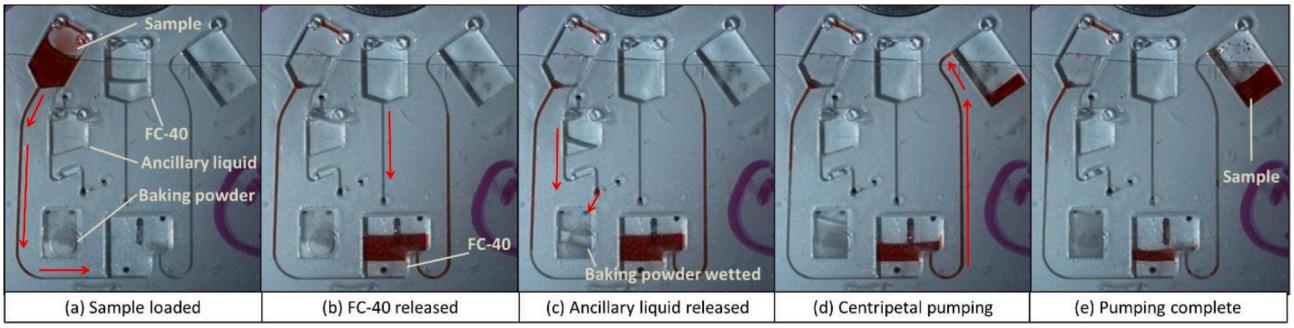

Fig. 2. Frame sequence of centripetal pumping according to Fig. 1. (a) After loading, the sample is centrifugally pumped into the outermost chamber. (b) Under the impact of the centrifugal field, the transparent, high specific-density liquid FC-40 is layered below the aqueous sample where it effectively seals the inlet channel. (c) Upon opening of the DF in the outermost chamber, ancillary liquid progresses into the chamber holding the dry baking powder. (d–e) The resulting production of CO_2 drives the sample up the radially inbound channel. Movie 1 in ESI demonstrates pumping of a single sample. Movie 2 shows pumping a single sample radially inwards and outwards three times. A prior version of this figure was published in (Kinahan et al., 2015b) and is adapted here with kind permission of IEEE.

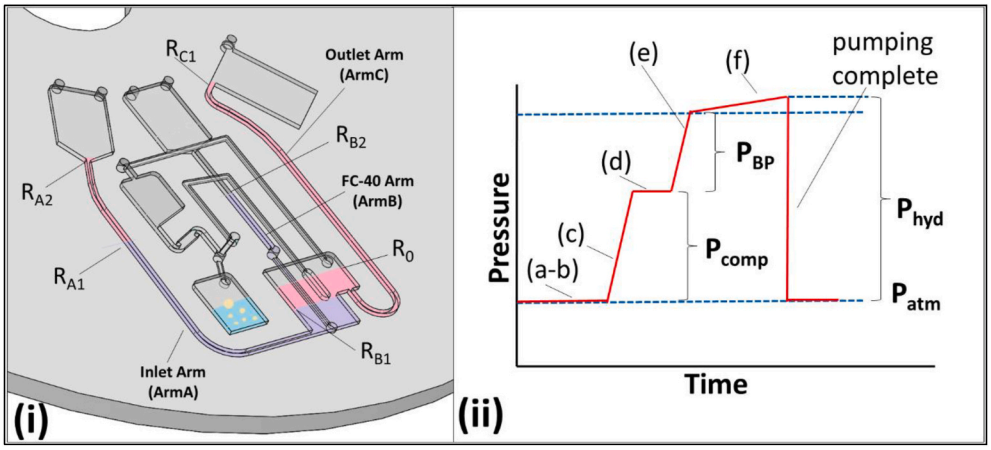

Fig. 3. (i) Detailed schematic showing the typical position of liquid levels at the onset of chemically powered centripetal pumping. The capital letters A, B and C designate the sample inlet arm, the FC-40 arm, and the outlet arm, respectively. (ii) Representation of gas pressure in the sealed pneumatic chamber. The letters in the inset refer to the panels shown in Fig. 1. Briefly, (a–b) refers to the entry of sample into the pumping chamber where the outlet is not yet sealed (no pressure increase), (c) to the release of FC-40 which seals the pumping chamber and pushes the liquid into it thus leading to an increase in pressure ($P_{\Delta V}$), (d) to the time required to open the event-triggered DF valve, (e) to the release of CO_2 from baking powder until the outlet liquid reaches the point R_{C1}, and (f) to the sample pumped into the sample collection chamber. The increasing gas volume moves the gas-liquid interface (R_0) radially outwards the sample is pumped from the chamber. Upon completion of pumping, there is a stepped decrease in gas pressure once the pumping chamber is vented to atmosphere. A prior version of subfigure (i) was published in (Kinahan et al., 2015b) and is adapted here with kind permission of IEEE.

pressure in the pneumatic chamber is determined by the height of this liquid column. With the release of CO_2 the pressure in the pneumatic chamber rises past the centrifugally induced hydraulic pressure

$$p_{\Delta V} + p_{BP} > p_{ArmC}$$

in the arms. As the liquid plug is effectively acts as a piston, this pressure increase displaces the liquid radially outwards and a droplet of liquid breaks off at R_{C1}. Thus, the release of CO_2 predominantly triggers an increase in the gas volume; and hence displaces the liquid radially inwards. The increasing pressure moves the location of R_0 radially outwards as pumping occurs; this results in an increase in overall pumping pressure in the chamber (Fig. 3).

The basic pumping mechanism can be advanced by arranging a number of these pumping modules in series. This concatenation is enabled using the FC-40 liquid as a valving mechanism, effectively plugging the inlet channel and preventing the backflow of sample (Fig. 4, see also Movie 2 in ESI).

3.2. Check-valve with just FC-40 (no DF valves)

It should be noted that in the approach described above (reciprocal pumping), the FC-40 is released, via a DF, after the sample liquid reaches the peripheral chamber. However, it was identified that the FC-40 could be placed, blocking the inlet to the pumping chamber, and, during sample loading, the centrifugally induced hydrostatic pressure generated in the inlet arm would exceed the induced back-pressure caused by FC-40 in the pumping chamber; this difference in hydrostatic pressures would allow the liquid to flow past the FC-40 and into the pumping chamber. Thus, the FC-40 can act as a gas-tight check-valve during disc rotation. It should be mentioned that a similar displacement strategy has been used for droplet generation on the LoaD (Schuler et al., 2015). This check-valve approach is used to demonstrate centrifuge-pneumatic pumping (Section 3.3) and is shown in ESI Video 3.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.biosx.2021.100085

3.3. Centrifugo-pneumatic pumping

Alongside chemically induced pumping, the multi-pump structure shown in Fig. 4 was also used, without baking powder or dissolvable film valves, to centripetally pump liquids by a pure centrifugo-pneumatic mechanism. Here, rather than rotating at a constant spin-rate per the method described in Section 3.1, the disc spin-rate is changed to activate the pumping mechanism. This approach used the immiscible FC-40 as a non-return valve preventing liquid back-flow. A similar pumping scheme, centrifugo-dynamic pumping, has been demonstrated previously by Zehnle et al. (2012). However, in their approach, the pumping efficiency was broadly determined by the ratio of fluidic resistances of the inlet and outlet channels. Their system acts with optimum efficiency based upon hydrodynamic pumping; where the rapid release of energy induces most liquid to flow through the channel of lower hydraulic resistance.

In our approach, the FC-40 is pre-loaded into the pumping chamber and blocks the inlet. Rapidly accelerating the disc to a high spin-speed pumps the sample into the pneumatic chamber through the immiscible liquid check valve. The outlet is sealed by the incoming liquid and so forms a dead-end pneumatic chamber. As further liquid is pumped into the chamber the air trapped in the dead-end chamber is further compressed. Next, the disc is slowly decelerated at 2 Hz s^{-1}, which reduces the centrifugally generated hydrostatic pressure and so the trapped air expands. As it expands, the air displaces the liquids radially inwards. Due to the density difference between the sample (water) and the FC-40, the liquid check-valve prevents the sample flowing back up the outlet channel so the sample is only displaced inwards through one channel; resulting in radially inward pumping.

It should be noted that, like Zehnle et al. (2012), our disc was accelerated as rapidly as possible. However, Zehnle et al. used a spin-profile where their disc was subject to rapid deceleration in order to maximise the performance of their system. This was because their routing mechanism was based on differences in the inlet and outlet microchannel flow resistances (which are more pronounced at high flowrates). In our approach, rapid deceleration disrupted our liquid check valve and prevented our pumping mechanism from functioning. However, slow deceleration of our disc (2 Hz s^{-1}) left the liquid check valve in place and resulted in efficient centripetal pumping of our sample (Video 3, ESI). The use of lower acceleration rates can be advantageous as it permits the use of a lower-powered motor (compared to methods that require rapid acceleration).

3.4. Reagent storage

The chemical pumping mechanism presented here can also be applied to reagent storage on the disc periphery by means of loading baking powder and water, separated by a rotationally actuated 'DF burst valve', into a sealed chamber with a reagent (Fig. 5). Below the DF valve actuation frequency, the chamber remains at atmospheric pressure,

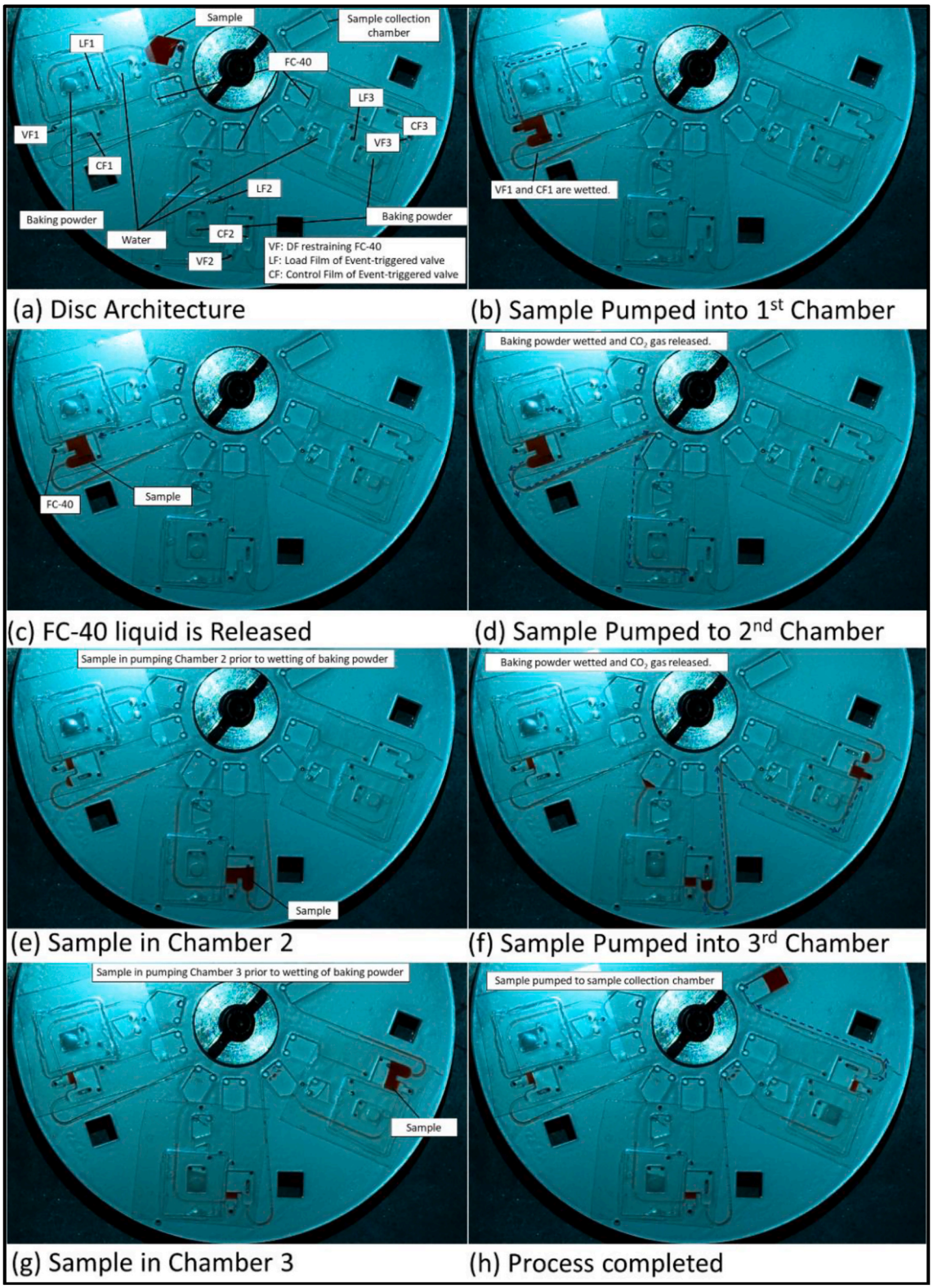

Fig. 4. Reciprocating pumping of a sample. (a) Upon spinning, the sample is centrifuged from its loading reservoir (b) to the first pumping chamber and wets the DFs. In this figure, Valve Film (VF) refers to the DF which restrains the immiscible FC-40 valving liquid. Control Film (CF) and Load Film (LF) refer to the component films of an event-triggered valve – dissolving the CF triggers the release of aqueous liquid through the LF as defined by Kinahan et al. (Kinahan et al., 2014b) previously. (c) FC-40 (clear) is released and blocks the inlet channel. (d) Ancillary liquid (water) is released, activates the baking powder to generate CO_2 which pumps the sample radially inwards and into pumping chamber 2 (e–h) The process is repeated through pumping chamber 2 and pumping chamber 3 until the sample enters a collection chamber located radially inwards. Note that this pumping occurs at a constant spin rate and is solely triggered by the entry of the liquid into the pumping chambers. A prior version of this figure was published in (Kinahan et al., 2015b) and is adapted here with kind permission of IEEE.

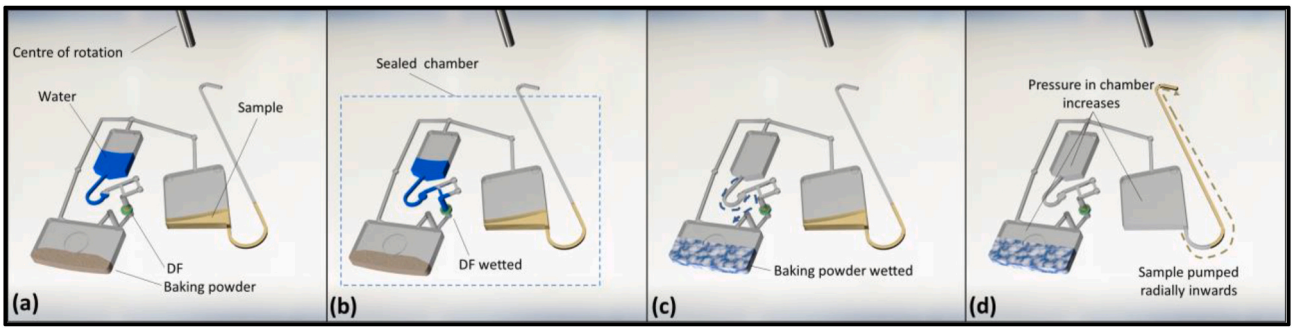

Fig. 5. Reagent Storage. (a) Storage of reagents/sample on the periphery of the disc and the baking powder in a dry state. The sample, water and baking powder are stored in a contiguous, sealed chamber from which there is only one outlet. The water is prevented from wetting the baking powder by a DF burst valve tuned to open at a specific frequency. (b) With an increase (or alternatively a pulse) in spin rate, the DF is wetted and (c) so the baking powder generates CO_2 which increases the pressure within the pumping chamber (d) to centripetally displace the sample.

storing the reagent at the disc periphery. Upon elevating the spin rate above the burst frequency of the valve, the baking powder is wetted to prompt the generation of CO_2 gas, and thus driving the liquid radially inwards. In this case, as there is no inlet channel, implementation of an FC-40 check-valve is not required.

3.5. *Liquid plug for routing*

Previously, Kinahan et al. (2014b) modified the event-triggered valving concept for liquid routing. In that work a DF valve was placed in a channel such that (upstream) liquid could not wet it due to trapped air. This DF was removed through contact with an ancillary liquid from the underside.

Here, we present an improved, more robust version of this approach where access to the DF from upstream liquids is blocked using the dense, immiscible FC-40 liquid, rather than a trapped air pocket (Fig. 6). This liquid plug prevents aqueous samples or washes from contact with the DF; this method is very robust due to the incompressibility of FC-40 and therefore, unlike the pneumatic approach previously described, does not possess an associated 'burst frequency'. Consequently, a robust spin protocol can be applied without risking premature opening of the routing mechanism.

4. System demonstration – solid phase DNA purification

To show the efficacy of reagent storage on the disc periphery, and the feasibility of the new routing mechanism presented in this paper, we apply this technology to nucleic acid purification (Boom et al., 1990). In Fig. 7 (a), the disc accommodates five reagent storage chambers in the outer region of the disc. Each chamber is composed of a reagent reservoir, a store of chemical energy (baking powder) and an ancillary liquid (water), which can trigger the release of CO_2. The water is separated from the baking powder by means of a one capillary and four DF burst valves which are geometrically tuned to open at different frequencies

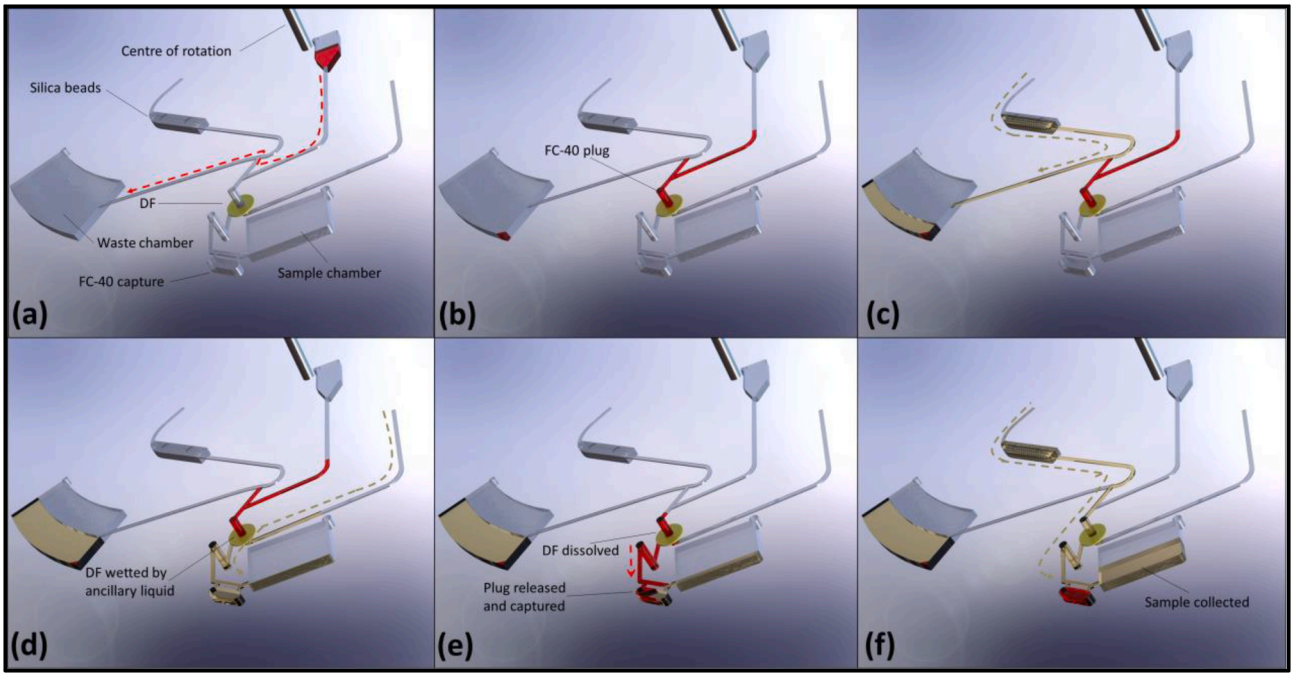

Fig. 6. Sample routing. (a–b) Initially a plug of dense, immiscible FC-40 liquid resides in front of a DF. This plug prevents water from contacting and thus dissolving the DF. (c) The solutions containing DNA and EtOH washes are both passing through acid washed silica beads and touted to waste in subsequent steps. (d) A small volume of (aqueous) ancillary liquid (DI water or elution buffer) contacts the routing DF from its underside. (e) The FC-40 plug is removed in a radially outer side pocket while the miscible ancillary liquid advances to the capture chamber where it will slightly dilute the collected sample. (f) The elution buffer washes through the beads, removes nucleic acid sample, and is then, following the path of least fluidic resistance, directed to the sample collection chamber.

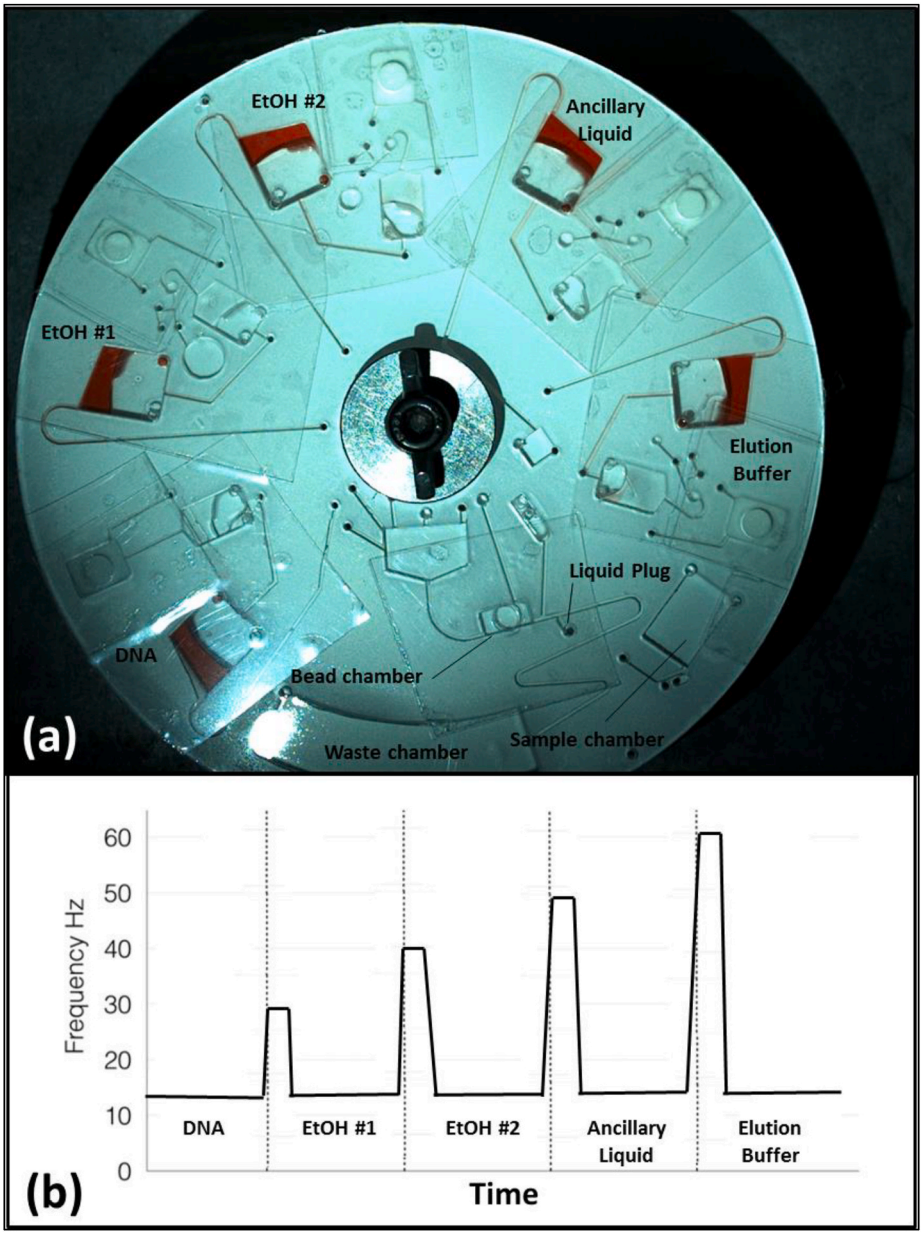

Fig. 7. Centrifugally automated DNA purification. (a) Disc architecture featuring five reagent storage chambers which are arrayed around the periphery of the disc to save precious real estate in the central region. These liquids are centripetally pumped and, except for the ancillary liquid, driven through acid-washed silica beads. (b) The pulsing of the spin protocol allows freely programmable timing of reagent release. Spinning at 15 Hz triggers the first (capillary) valve to pump the sample. The subsequent valves are opened by transient spikes in the spin rate to 30 Hz, 40 Hz, 50 Hz and 60 Hz, respectively. Once the DF is wetted, the spinning is reduced to 15 Hz during pumping to increase the residence time of the sample/washes with the beads.

(Fig. 7b); thus, the pumping of each reagent can be triggered by exceeding its individual burst frequency. Operation of this disc is shown in Video 4 (ESI).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.biosx.2021.100085.

As described in Section 2.3, the DNA standard is first diluted in a buffer containing a chaotropic salt. This results in DNA present in the buffer preferentially binding to the silica beads (solid-phase) when washed through our disc. This wash of DNA through the glass beads is followed by two EtOH washes (Section 2.3). Next, an ancillary liquid wets the underside of the routing DF to remove the liquid plug (Fig. 6). This FC-40 liquid element is trapped in a capture chamber. Finally, the elution buffer washes through the glass beads, removing DNA bound to the glass beads and transferring DNA to the sample collection chamber.

As described in Section 2.3, the purification efficiency is measured by comparing fluorescence from a DNA standard to this same DNA standard after it is processed on disc or processed through a commercial 'spin column' protocol. The purification efficiencies ($n = 3$) obtained from this disc was defined relative to the reference DNA (100% ± 3.9%). Using the commercial kit, the extraction efficiency was 60.6% ± 4.5% and using the disc the extraction efficiency was measured as 28.5% ± 8.6%. Therefore, relative to the 'gold-standard' commercial platform, the DNA recovery is 47%.

5. Conclusions and outlook

The chemical pumping mechanism presented in this paper expands the capabilities of the centrifugal platform. As the pump is only triggered by the presence of the sample, the structure operates widely independent of the spin rate. Thus, and unlike most previous implementations of centripetal pumping, we developed a module which can readily be inserted at any point of an on-disc workflow without affecting upstream and downstream processes. Additionally, this pumping scheme consumes comparatively little space and, due to pneumatic connecting channels, the ancillary liquid and baking powder can be located at arbitrary, e.g., also outer and thus more spacious regions on the disc.

Thus, unlike previous centripetal pumping schemes, the technique saves valuable central real-estate. The observed, minor loss of sample

during pumping (representing a lack of efficiency) is primarily linked to our present system design and prototyping methods, rather than representing a method-inherent limitation. While CO_2 can render aqueous samples acidic, in our platform this effect should be negligible as the pumping takes place over a relatively short time (~30 s–45 s) and the CO_2 can only diffuse through the liquid/gas interface.

The use of FC-40 as a 'plug' to selectively block channels also widens the options for centrifugal flow control. When applied to the baking-powder driven mechanism, the piston-like liquid element enables efficient pumping with minimal back flow. Indeed, as described in Section 3.3, this normally-open valve configuration allows efficient centripetal pumping based entirely on changes in the spin speed. With its normally-closed variant, efficient on-disc liquid routing by selective blocking of channels from aqueous liquids, and subsequent removal of the plug, is demonstrated. The use of this platform for reagent storage can be easily incorporated into a disc workflow by conventional burst valves. An additional advantage of this approach is that, on a system built around water dissolvable DFs, there is an open channel between the sample and its target location. Thus, this storage method can be applied to a wide scope of non-aqueous sample types such as ethanol (as demonstrated here) or oils.

These novel flow control strategies have been applied to multi-step SPE protocol for nucleic acid detection. The focus of this work was on demonstrating underpinning fluidic pumping, valving and routing technology. Future research might be directed towards improving the efficiency of the DNA purification towards comprehensive sample-to-answer automation of multiplexed bioanalytical assays. We identify two major changes which might make the purification more efficient; in the first the replacement of the column of acid-washed glass beads with a silica frit would increase the surface area for DNA binding and ensure that all samples flow 'through' the solid-phase rather than around it. In the second, assay performance might also be improved by the increasing the interaction (incubation) time of the sample with the solid-phase. This has been demonstrated (without centripetal pumping) using a siphon-controlled incubation structure (Henderson et al., 2021) and, using the same assay as described here, resulted in measured efficiency of ~58% compared to the ~28% reported here. However, it should also be noted that the primary focus of SPE is to remove agents that inhibit nucleic acid amplification; by their nature PCR, LAMP, RPA and other amplification chemistries can be used to detect the presence of with very low (but pure) concentrations of DNA.

CRediT authorship contribution statement

David J. Kinahan: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Robert Burger:** Conceptualization, Methodology, Writing – review & editing. **Daryl Lawlor:** Methodology, Investigation, Writing – review & editing. **Philip L. Early:** Methodology, Investigation, Writing – review & editing. **Abhishek Vembadi:** Methodology, Investigation, Writing – review & editing. **Niamh A. McArdle:** Methodology, Investigation, Writing – review & editing. **Niamh A. Kilcawley:** Methodology, Investigation, Writing – review & editing. **Macdara T. Glynn:** Methodology, Investigation, Writing – review & editing. **Jens Ducrée:** Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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