TWO-DIMENSIONAL PAPER\textsuperscript{1} NETWORKS: MULTI-STEP FLUIDIC PROGRAMMING USING A FLUID SOURCE WELL & SHAPED PAPER

Barry R. Lutz, Philip Trinh, Cameron Ball, Elain S. Fu, Paul Yager

Department of Bioengineering, University of Washington, Seattle, WA, USA

ABSTRACT

Most laboratory assays take advantage of multi-step protocols to achieve high performance, but conventional paper-based tests (e.g., lateral flow tests) are generally limited to assays that can be carried out in a single fluidic step. We present a method to automate multi-step chemical delivery in a simple paper device via a single user activation step. A fluid well is used as a buffer source to feed a paper network; wicking causes the fluid level in the well to decrease, and different paper legs are disconnected in a timed sequence to allow sequential timed delivery of fluids to a detection zone.

KEYWORDS: paper diagnostics, point of care diagnostics, low resource settings, developing world

INTRODUCTION

Devices fabricated from paper or other porous material provide an inexpensive “microfluidic” platform for chemical analyses or medical diagnostics without the need for pumps. The classic example is the lateral flow test (LFT), which uses a single strip of paper with dried reagents to provide visual detection in an easy-to-use format. A new class of paper-based tests uses branched channels to split a sample into zones with different testing chemistries [1-6]. However, this approach has thus far been limited to tests that can be carried out with a single fluid delivery step, just as with conventional LFTs. In contrast, nearly all laboratory assays take advantage of multi-step protocols to achieve high performance. We are developing timing mechanisms that are introduced into paper to “program” autonomous multi-step assays [7-9]. Arbitrarily complex multi-step processes can be created by combining two functions: 1) controlling the arrival time of individual reagents to a detection zone, and 2) controlling the shut off time of each reagent. Here, we present a method that uses paper strips of different lengths inserted in a volume-limited well to allow single-step user activation of an autonomous multi-step fluidic sequence.

THEORY

The wicking of fluid into paper strips can be described by the classic Washburn equation describing 1D wicking in porous media: \( L = (Wt)^{1/2} \), where \( L \) is the distance fluid has travelled, \( t \) is time, and \( W \) is a parameter representing the wicking properties for the fluid and material. When multiple wicking strips are inserted into a common well, the fluid level drops, and this effect can be used to disconnect legs that are inserted to different depths. For a given fluid and material, the Washburn coefficient \( W \) can be measured directly by applying the Washburn equation to wicking measurements, and liquid capacity \( C \) can be measured by weighing saturated materials. The volume uptake can be approximated using the fluid capacity of strips as \( V = C\mu L \). For 5 mm wide nitrocellulose strips used here, \( W = 9.5 \text{ mm}^2/\text{s} \) and \( C = 0.6 \mu \text{L/mm} \).

EXPERIMENTAL

To reduce evaporation effects, the devices were enclosed in plastic housings. The housings and fluid wells were fabricated from a PMMA sheet (2.5 mm thick) and 0.25 mm thick Mylar film with adhesive coating on both sides (10 mil, Fraylock Inc, San Carlos, CA); materials were cut to shape by a CO\textsubscript{2} laser cutter (Universal Laser Systems, Scottsdale, AZ), assembled by hand, and pressed to seal the adhesive. Nitrocellulose membranes (Mylar-backed HiFlow Plus 135, HF13504, Millipore, Billerica, MA) were cut using the laser cutter. Legs were mounted on an adhesive Mylar backing such that their ends protruded different lengths. Food coloring was dried on cellulose pads (C083, Millipore) to mimic dry assay reagents; for convenience of fabrication and alignment, the nitrocellulose was scraped from the Mylar backing such that the pads bridged two nitrocellulose sections. Fluid fronts were tracked manually from webcam videos recorded at one frame per second (Logitech, Fremont CA) using ImageJ[10].

RESULTS AND DISCUSSION

Figure 1 shows the position of fluid fronts as a function of time for “legs” immersed to different depths in a common fluid well. As the fluid level drops, each leg breaks contact with the source fluid at a different time; thus fluid delivery to each leg is shut off in a timed sequence. Data are plotted in the form of the Washburn equation, \( L^2 = Wt \). As expected, all legs follow the Washburn line \((W = 9.5 \text{ mm}^2/\text{s})\) until they are disconnected from the dropping fluid surface. The fluid front advances somewhat after the disconnection due to a period of equilibration of the fluid into the dry paper. Nevertheless, the fluid delivery to each leg is shut off in a well-controlled and timed sequence. The shut-off times for fluid delivery were \(28 \pm 13 \text{ sec (47%)}, 66 \pm 20 \text{ sec (31%)}, \) and \(178 \pm 68 \text{ (38%)}. \)

\textsuperscript{1}We use the term “paper” broadly to describe fibrous materials used as wicking materials and assay substrates in diagnostics. Nitrocellulose used here is not technically classified as paper.
The combination of a 2DPN with a volume-limited well provides the functions needed to program autonomous multi-step fluidic processes: 1) controlled arrival time of reagents to a detection zone, and 2) controlled shut off time for each reagent. Figure 2 shows a 2DPN housing with a fluid well. Device operation involves two steps: 1) filling the well with buffer, and 2) inserting the 2DPN. Figure 3 shows the automated delivery of colored fluids after activation. The dried dyes are rehydrated, and each fluid is staged in the main channel. The dropping buffer level disconnected three legs, and the fourth leg remained connected allowing multiple fluids to be delivered to the main leg in a timed sequence with a total time that is appropriate for many common assays (~30 minutes total). Compared to LFTs that are capable of only a single delivery step, the 2DPN can provide multi-step delivery of reagents needed for more sophisticated assays.

We previously showed that three legs can be used to deliver reagents for an amplified immunoassay using a different shut-off method, i.e. individual fluid source pads [7]. The well method described here requires the device to stand (roughly) upright, but the buffer loading step is simplified. 2DPN multi-step programming could be combined with branched paper devices, and it is compatible with other fabrication methods [1-6]. In addition, alternative methods for flow control [11] and time indicators [12] could be integrated into 2DPNs that use volume-limited pads or wells for timed delivery.

To achieve shut off times appropriate for many assays (minutes), the design required minimizing the well cross-sectional area (thin and narrow when viewed from the top). In small wells, surface tension forces are significant, which can lead to capillary bridges between the paper and well surface and/or prevent leveling of the fluid surface by gravity. We found that reproducibility of shut off timing was improved by adding surfactant (we normally use surfactant in our assay buffers) and by using pointed leg tips shown in Fig. 1. In addition, we found that a “regulating wick” can be added to increase the rate that liquid is drawn from the well, which allows legs to be spaced further apart and well dimensions to be increased if needed. For example, a 1cm wide cellulose regulating wick with a high fluid capacity ($C=7.3 \ \mu$L/mm) and a fast wicking rate ($W=37 \ \text{mm}^2/\text{s}$) draws fluid ~22x faster than a nitrocellulose strip (ratio of $C \cdot W^{1/2}$ for the two materials).

Figure 1. Fluid shut off from a volume-limited source well. A) Nitrocellulose legs were immersed to different depths into the well (1.2, 1.5, 1.9, and 2.2 mm). B) As fluid was wicked, each leg broke contact with the fluid in a timed sequence to provide programmed shut off. C) Plot of the fluid front position in each leg as a function of time. The fluid front in each leg follows the Washburn behavior ($L^2=Wt$) until it breaks contact with the fluid in the well (indicated by arrows and white circles). The fluid front migrates somewhat after shut off due to relaxation of the fluid into the dry membrane. Pointed leg tips provide more reproducible fluid break-off than blunt-ended legs. Shut off times for repeated experiments were $28\pm13$ seconds, $65\pm20$ seconds, and $178\pm68$ seconds for legs 1, 2 and 3, respectively ($n=3$). Fluid: Tris-buffered saline with 0.01% Tween 20 (TBST) and red food coloring. Legs: 0.5 cm wide, $W=9.5 \ \text{mm}^2/\text{s}$. Well dimensions: 3 cm wide x 0.25 mm thick (10 mil Mylar spacer). Fluid fronts were tracked manually from webcam videos recorded at one frame per second using ImageJ.

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CONCLUSIONS

Laboratory assays normally use multi-step reagent sequences to achieve high performance. The combination of a 2DPN with a volume-limited fluid source provides the two basic functions needed to program timed processes for autonomous multi-step diagnostics: 1) controlled arrival time of reagents to a detection zone, and 2) controlled shut-off time of each reagent. This approach could enable a wide variety of multi-step laboratory assays to be converted into simple point-of-care devices that have high performance and are easy to use.

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CONTACT
Barry Lutz, blutz@uw.edu. Elain Fu, efu@uw.edu. Paul Yager, yagerp@uw.edu.