Fluidic "Timers" for Paper-Based Microfluidic Devices

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Supporting Information

Materials

Paper

Whatman Chromatography Paper No. 1 (200 mm × 200 mm) was used without further adjustment of size.

Aqueous dyes for revealing the flow of fluids within μPADs

Synthetic food dyes (Assorted Food Colors & Egg Dye; Wal-Mart brand) were used to give colorimetric responses and to track the distribution of fluids within a device. The synthetic food dyes contain the following components: RED 40 (disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid.), BLUE 1 (disodium salt of ethyl [4-[*p*-[ethyl(*m*-sulfobenzyl) amino]-a-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadien-1-ylidene](*m*-sulfobenzyl)

ammonium hydroxide inner salt plus *p*-sulfobenzyl and *o*-sulfobenzyl salts), YELLOW 5 (trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenylazo]-1H-pyrazole-3-carboxylic acid), and GREEN (a 1:1 mixture of YELLOW 5 and BLUE 1). The dyes were used as 1:5 mixture of dye–distilled water.

Methods

Designing and printing of microfluidic channels in paper

CleWin® (PhoeniX Software, The Netherlands) was used for designing patterns in paper and adhesive tape. Designs were saved as PostScript files, which were converted into PDF files for printing. A Xerox Phaser 8560N color printer was used for depositing solid wax onto paper in defined patterns according to the procedures reported by Carillho et al.[1] Printing quality was set at the highest resolution for photo quality printing. Printed papers were placed on a hot plate set at 150 °C for two minutes. During this time, the wax ink penetrated through the paper in the z-direction to create hydrophobic barriers within the paper. Solid inks are composed of a mixture of hydrophobic carbamates, hydrocarbons, and dyes; when combined, these ingredients melt at 120 °C. The patterned paper was cooled to room temperature, and was ready for further processing after 10 s.

Patterning tape

An Epilog Laser (Epilog Mini, 45 W) CO_2 laser cutter was used to cut holes in double sided adhesive tape (ACE plastic carpet tape 50106). The patterns for these holes were designed in CleWin®, as described previously.[2]

Fabricating meters

Paraffin wax from Sigma Aldrich was used as received. Paraffin wax is a mixture of hydrocarbons obtained from petroleum fractions. The paraffin wax used in these experiments had a melting point of 58–62 °C. Hexanes (Sigma Aldrich) was used to dissolve the paraffin wax; solutions were sonicated for up to 10 min to facilitate complete dissolution of the wax into hexanes. Solutions (0.4 μL; concentrations ranging from 1–55 mg mL^{-1}) of wax in hexanes were deposited (using a micropipette) onto hydrophilic regions of paper that were 2.4-mm diameter × 0.18-mm thick. Once the hexanes had evaporated (ca. 30 min), another 0.4 μL of the same wax solution was deposited on the bottom of the same hydrophilic region (the opposite side of the paper). The paper was air-dried at room temperature for 1 h in a chemical fume hood.

Fabricating 3D μPADs

3D μPADs were assembled using procedures similar to those described by Martinez et al.[2] The holes in the tape were filled with Whatman Chromatography Paper #1 that had dimensions equal to the size of the holes. The assembled 3D μPADs were compressed with a rolling pin by passing the rolling pin over the devices three times with pressure approximately equal to that required for rolling dough.

Measuring contact angles

Wax solutions were deposited using procedures similar to those for fabricating meters. Solutions (100 μL; concentrations ranging from 1–50 mg mL^{-1}) of wax in hexanes were deposited (using a micropipette) onto circular sections of Whatman Chromatography Paper No. 1 (90-mm diameter). Another 100 μL of the same wax solution was deposited onto the opposite side of the same area of paper after hexane from first deposition had completely evaporated. The contact angles of the dried papers were measured using an instrument manufactured by First Ten Angstroms Inc. (Portsmouth, VA). A drop of liquid (distilled water, bovine serum, or bovine serum albumin, 10 μL) was deposited on the top of each sample of paper using an automatic syringe pump. Contact angles were measured when the drop of water first touched the top of the paper.

Measuring Gurley Porosity Values

These measurements were conducted by Integrated Paper Services, Inc. Each sample was measured eight times.

Glucose assays

a) Blue colorimetric assay:

The glucose assay reported by Wang et al. produces a blue color in response to glucose,[3] and we followed this procedure with slight modifications. Briefly, this assay uses 4-chloro-1-naphthol (4-CN) and *N,N*-diethyl-*p*-phenylenediamine dihydrochloride (DEPDA) as substrates for horseradish peroxidase (HRP). In the presence of HRP and H_2O_2, these substrates react to form a water insoluble blue indamine dye. Solutions containing 10 mM

4-CN in ethanol and 10 mM DEPDA in 20% MeCN–H_2O were prepared in separate containers and then mixed in 2:1 v/v ratio, respectively. Prior to running the assay, we pre-deposited 0.2 μL of the mixed solution onto a 2.4-mm diameter detection zone on the bottom layer of patterned paper. Once dry, 0.6 μL of a second reagent solution containing glucose oxidase (100 U/mL) and horseradish peroxidase (5 U/mL) in distilled water was added to the same detection zone and dried in air for 20 min. Pre-deposited reagents were stabilized by the addition of 0.2 μL of a 0.3 M trehalose solution (prepared in 0.1 M phosphate buffer, pH 6.0) to the same 2.4 mm diameter detection zone. The paper was dried in air for 10 min before it was incorporated into a μPAD. On the top layer of the 3D μPAD, 10 μL of a 10 mM solution of glucose in distilled water was added using a micropipette. In 2D μPADs, 10 μL of this sample was wicked into the device from the side of the device. The assays were conducted at 20 °C and 70% humidity. The assay was read when the fluidic timer indicated that the assay was complete.

(b) Green colorimetric assay:

The glucose assay reported by Erel et al. produces a green color in response to glucose,[4] and we followed their procedure with slight modifications. Prior to running the assay, we pre-deposited 0.2 μL of a solution containing 40 mM *ortho*-dianisidine dihydrochloride and 91 mM 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) in deionized water. This solution was deposited into a 2.4-mm diameter detection zone on a layer of a patterned paper. Once dry, 0.6 μL of a second reagent solution containing glucose oxidase (100 U/mL) and horseradish peroxidase (5 U/mL) in distilled water was added to the same detection zone and dried in air for 20 min. Pre-deposited reagents were stabilized by the

addition of 0.2 μL of a 0.3 M trehalose solution (prepared in 0.1 M phosphate buffer, pH 6.0) to the same 2.4-mm diameter detection zone. The paper was dried in air for 10 min before it was incorporated into a μPAD. On the top layer of the 3D μPAD, 10 μL of a 10-mM solution of glucose in distilled water was added using a micropipette. The assays were conducted at 20 °C and 70% humidity. The assay was read when the fluidic timer indicated that the assay was complete.

Measuring the color intensity of glucose assays

Videos and images of the wicking process were recorded with a SONY Handycam® camcorder. The histogram function in Adobe®Photoshop® was used to measure the luminosity produced in the detection zones during the glucose assay.[5] Luminosity (L_{exp}) decreases as the intensity of color increases in the detection zones. Therefore, we normalized the luminosity measurements by subtracting L_{exp} from background luminosity (L_{paper}) for the white detection zones, and then dividing this value by the luminosity value obtained at the end of an assay (L_{max}).

Measuring the fluorescent intensity of FITC-BSA and Fluorescene

The 3D μPAD was carefully disassembled immediately after the wicking process of the fluorescently-labeled protein solutions had finished. Each layer was placed under UV lamps at a distance of 5 cm from the lamp. Images of each layer were taken with a Nikon photo camera. The luminosity that was present in the detection zones was determined using the methods described for measuring the luminosity of glucose assays.

Turn-on time for colorimetric fluidic timers

We designed a ring-shaped stencil using CleWin®, and used an Epilog Laser (Epilog Mini, 45 W) to cut the pattern into a transparency film (3M). We used a crayon to draw a circle of red wax around the inside edge of the observation zones. Prior to drawing, the crayon was sharpened to provide lines with a thickness of ca. 1-mm.

Preparing chemiluminescent fluidic timers

A chemiluminescent reagent solution was prepared according to a procedure described by Sigma–Aldrich,[6] but with slight modifications. For example, the detection zone in the 7th layer of paper was pre-deposited with 0.6 μL of a solution containing 70 mM sodium perborate, 106 mM sodium carbonate, and 11 mM luminol dissolved in distilled water. This layer was dried in air prior to incorporation into the device. The timer detection zone on the bottom layer of the 3D μPAD was pre-deposited with 0.6 μL of a 10 mM $K_3Fe(CN)_6$ solution in distilled water. This layer of paper was dried in air prior to assembling into the 3D μPAD.

Audible fluidic timer with glucose assay

Blue colorimetric assay reagents were prepared and pre-deposited on detection zones on the bottom of patterned paper. A 6 M sodium chloride solution in water (10 μL) was pre-deposited onto a separate hydrophilic region (i.e., a 6-mm diameter circle at the bottom of patterned paper). The paper was dried in air for 20 min or until the water was completely evaporated. Circuit lines on the bottom of patterned paper were drawn with an acrylic-based silver conductive pen.[7–8] The conductive lines were dried in air for 1 h to cure the

wires. A buzzer (with an internal drive) and a silver oxide battery (1.55 V) were attached to the circuit using a cyanoacrylate-based adhesive. The paper was dried in air for 5 min before it was incorporated into a μPAD. A 10 mM solution of glucose in distilled water (15 μL) was added to the top layer of the 3D μPAD using a micropipette. We used a sound level meter to measure the intensity of the sound pressure produced by the buzzer. The sound level meter was positioned at a distance of 10 cm from the 3D μPAD at the time of the measurement.

Results

Dimensions of Devices

The following schematics describe in detail the dimensions of the 3D μPADs shown in Figures 1–4.

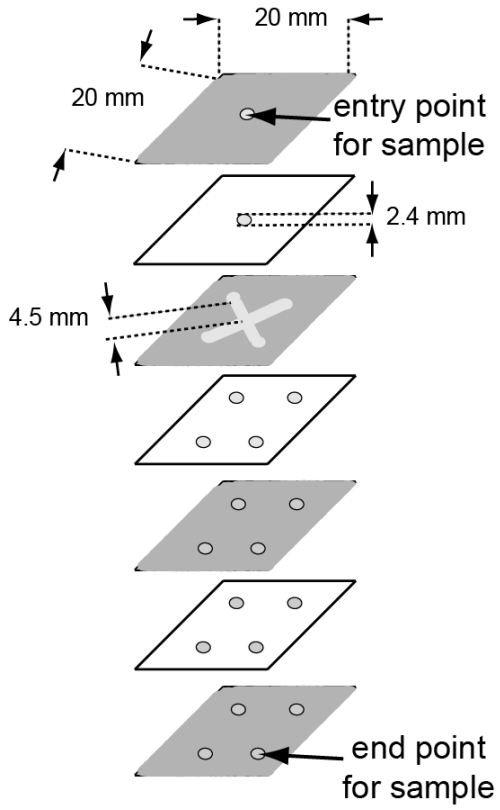


Figure S1. Dimensions of the device described in Figure 1. The length of the 4.5-mm channel in the image begins at the bottom of the circular end region of the channel and extends to the center of the point where the channels cross. The devices shown in Figures 5 and S10–12 possess nearly identical dimensions, with the exception that the number of layers is different in each device.

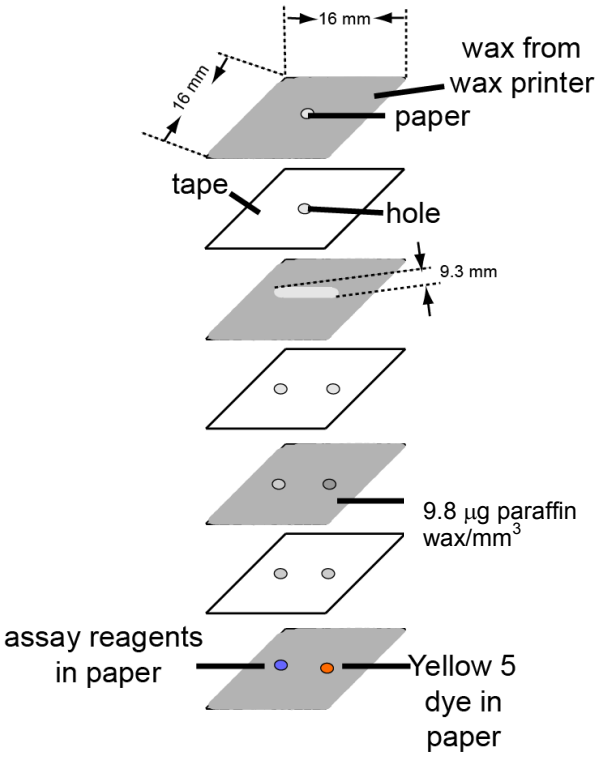


Figure S2. Dimensions of the device described in Figure 2.

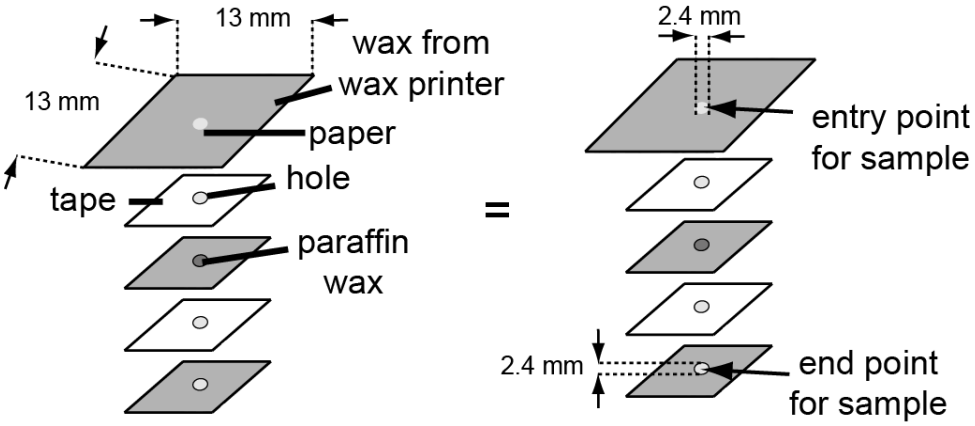


Figure S3. Dimensions of the device described in Figure 3.

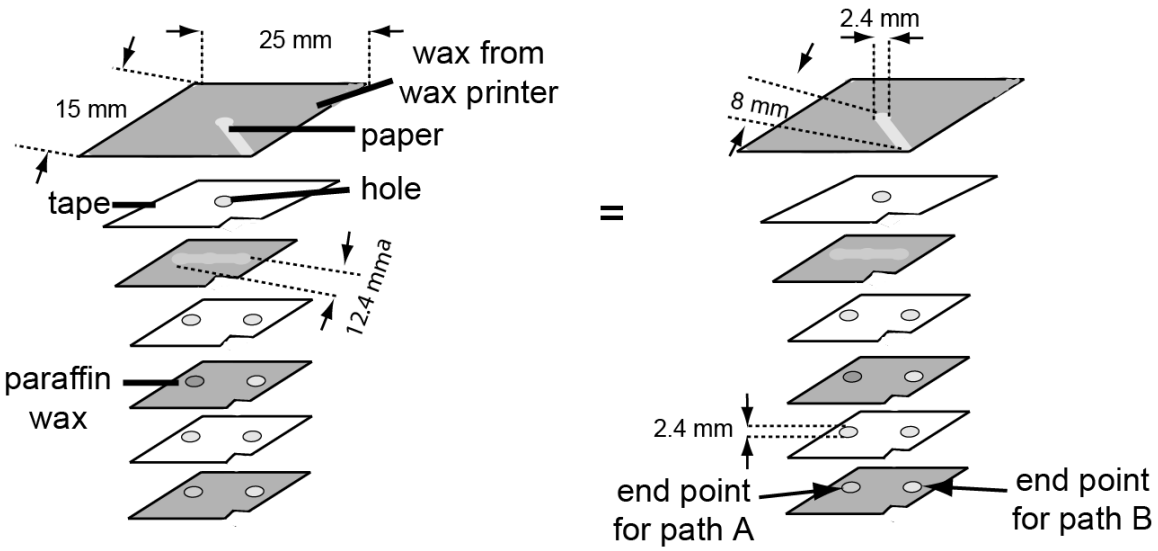


Figure S4. Dimensions of the device described in Figure 4.

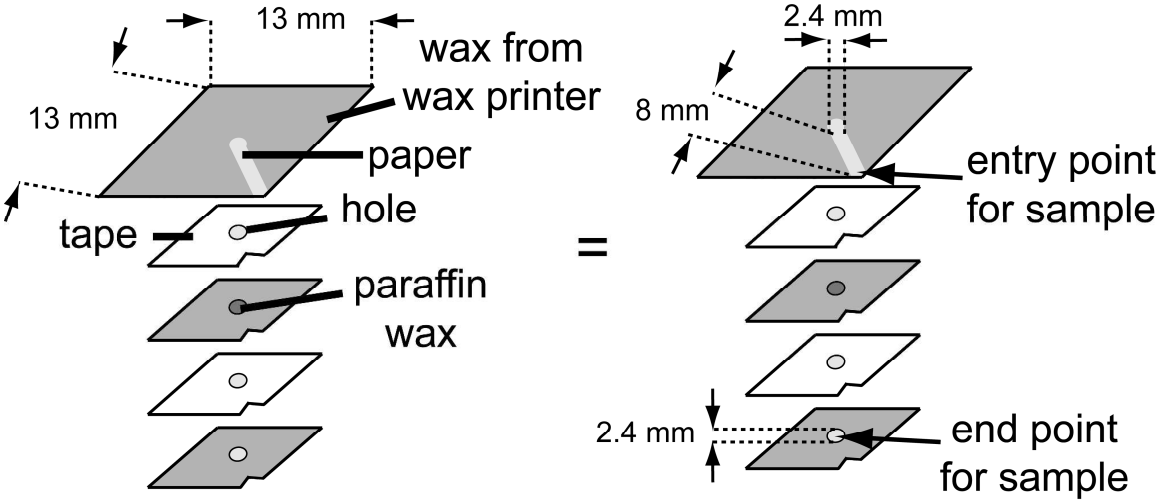


Figure S5. Dimensions of the devices used in Table 2.

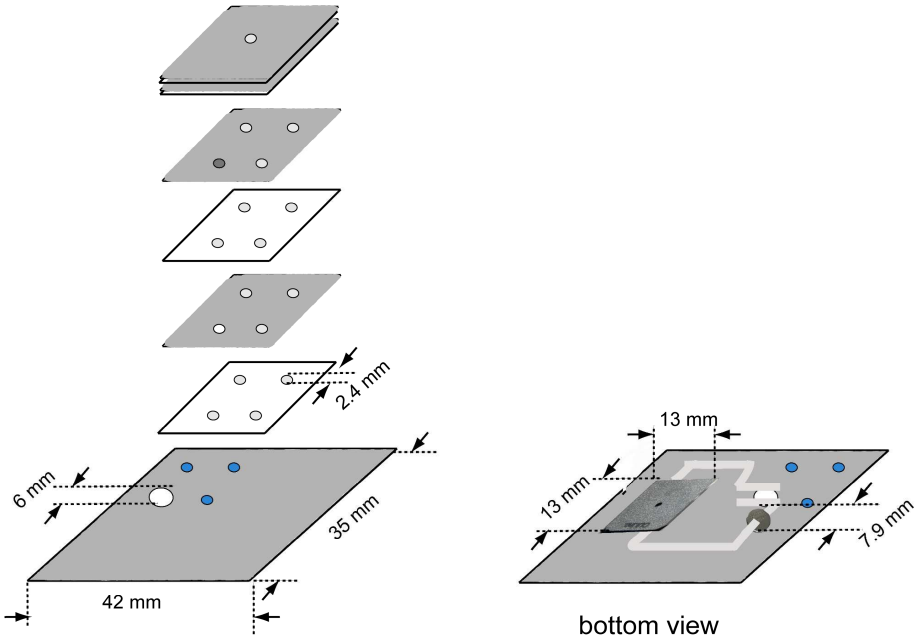


Figure S6. Dimensions of the device described in Figure S12.

Cross Sections of Timers: Analysis of wicking rate using the Washburn Equation

The graph in Figure 5a was generated from data points obtained using the following device and images.

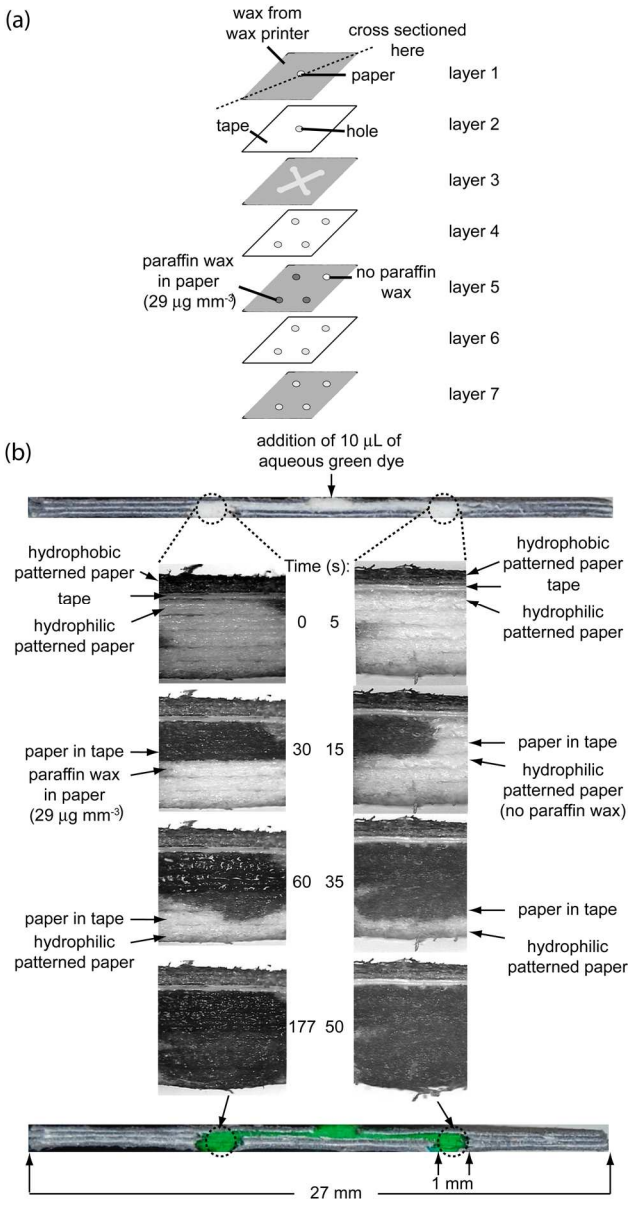


Figure S7. Comparison of fluid distribution rates through a 3D μPAD with and without a meter. (a) Expanded top view of the device with a dotted line that indicates where the device was cut in half. (b) Black and white time-lapsed photographs showing the cross-section of the device in (a). For these photographs, 10 μL of an aqueous solution of green dye in water (1:5 dilution of green food coloring to distilled water) is shown distributing through two conduits within the device. The left conduit contains a meter (29 μg paraffin wax per mm^{-3} of paper)

and the right conduit does not. The images were acquired at 25 °C and 40% humidity, and the green aqueous solution appears in the expanded images as a darkening of the white paper.[9]

Effects of the Type of Fluid on the Performance of the Timer

Qualitative observations of the wicking behavior of serum and solutions of BSA in water are shown below.

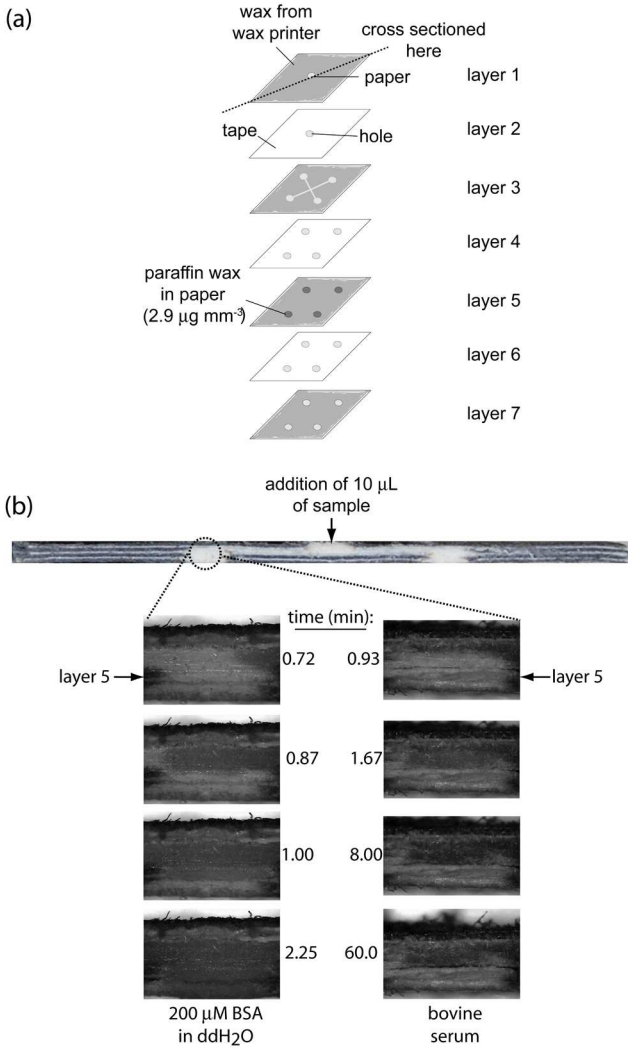


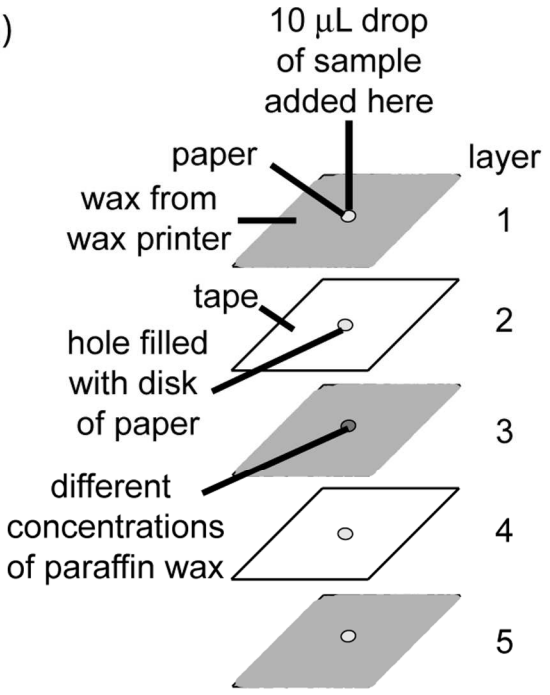
Figure S8. Effect of the type of fluids on the fluid distribution rates through a 3D µPAD that contains a meter. (a) Expanded top view of the device with a dotted line that indicates where the device was cut in half. (b) Black and white time-lapsed photographs showing the cross-

section of the device in (a). Cross-section images are shown of two independent devices that distributed 200 μM BSA or serum (bovine) through a fluidic timer. For these photographs, 10 μL of an aqueous solution of green dye in BSA solution (1:10 dilution of green food coloring to the BSA solution) is shown distributing through one conduit within the device. Serum was used without further modification of its yellow color. The left conduit contains a meter (2.9 μg paraffin wax per mm^{-3} of paper). The images were acquired at 25 °C and 40% humidity, and the fluids appear in the expanded images as a darkening of the white paper.

Testing Whether Proteins Accumulate on the Wax Meter

Observations of the wicking behavior of fluorescein-labeled BSA (FITC-BSA) in fluidic timers are shown below.

(a)



(b)

	average fluorescent intensity (arbitrary units)[a]		
layer	[wax] in layer **3** (µg mm^{-3}): 0	1.9	12.5
1	----	----	----
2	48 ± 7	53 ± 6	60 ± 7
3	50 ± 7	44 ± 6	35 ± 3
4	----	----	----
5	53 ± 5	45 ± 7	17 ± 1

[a] Measured as luminosity. N = 8

Figure S9. Distribution of FITC-BSA in layers within a fluidic timer. (a) Device used to image the location of FITC-BSA after it wicked through a fluidic timer. (b) Table of fluorescence intensity versus layer of the device.

Other Configurations of Fluidic Timers

(a) Multiple fluidic timers per device. Running more than one assay on a single diagnostic device often is complicated by the different rates of each assay: some assays proceed faster than others. The current approaches to running multiple quantitative, time-based assays on a single paper-based device involve either (i) using more than one external timer to track the individual assays, or (ii) altering the quantity of reagents in the assays to synchronize the rates of the assays. The latter option is possible for some assays,[5] but not for others, and the former option requires a level of rigor beyond what is possible to implement easily in the developing world. Fluidic timers (Figure S10) can be used to address the issue of timing multiple simultaneous assays: one fluidic timer can be incorporated for every assay on a device.

Figure S10 shows an example of a device that runs two assays with different time durations: the device has two separate fluidic timers (a 3.5 min timer and a 10 min timer) and two assays (in this case, both assays measure the levels of glucose, but one assay runs longer than the other[10]). The 3.5 min assay for glucose uses glucose oxidase, horseradish peroxidase, *o*-dianisidine, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), and produces a green indamine dye in the presence of glucose. The 10 min assay uses the same enzymes, but incorporates diethyl phenylenediamine, 4-chloro-1-naphthol, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), and produces a blue indamine dye in response to glucose. The 3.5 min timer reveals Red 40 in the bottom layer of the μPAD when 3.5 min has passed, and the 10 min timer reveals Yellow 5 after 10 min has passed. A movie of this process is available in the Supporting Information.

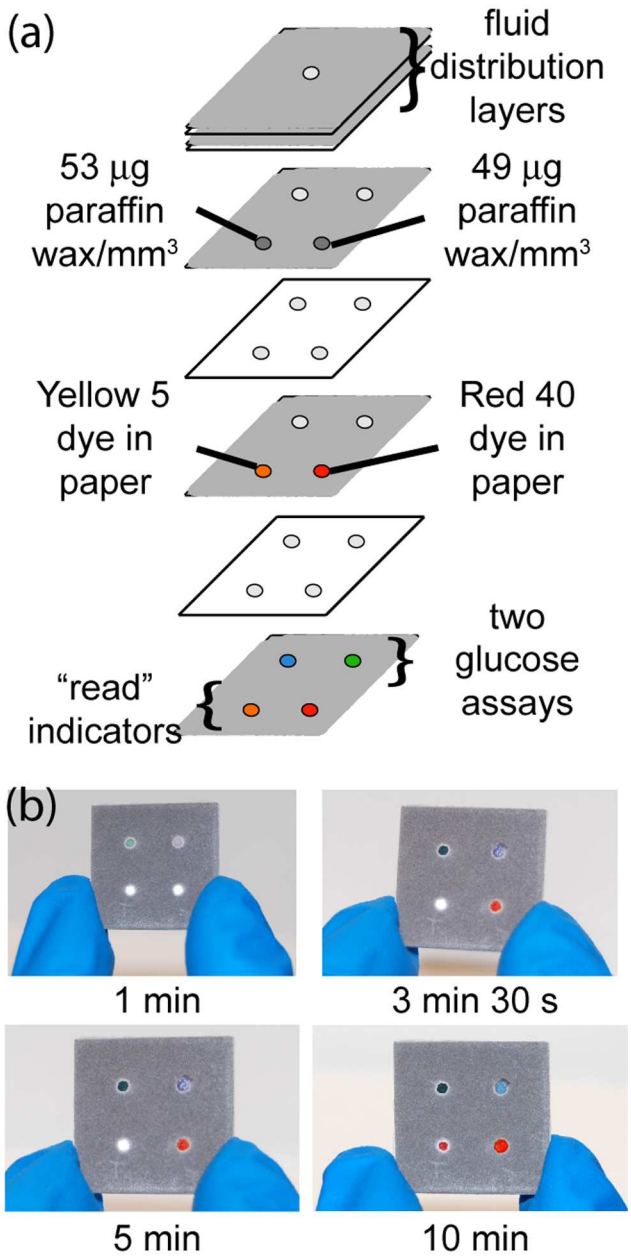


Figure S10. A 3D µPAD that contains two fluidic timers and two assays. (a) Expanded view of the 3D µPAD showing the distribution of reagents in each layer. Red 40 and Yellow 5 (1 µL of each) were pre-deposited into separate regions in the 7th layer as 1:5 dilutions of food coloring–distilled water prior to assembling the device. (b) Time-lapsed photographs of the back (i.e., bottom) of the device after adding 10 µL of a 10 mM solution of glucose in ddH_2O at 23 °C and 40% humidity.

(b) Chemiluminescent fluidic timers. In some locations in the developing world, electricity is intermittent, or non-existent, and although colorimetric fluidic timers provide unambiguous stop times during the daylight, they cannot be used for running time-based assays in the dark. Obviously, diagnoses must be made at night as well as during the day, so there are compelling reasons to develop inexpensive diagnostic devices that function in daylight and at night.

Figure S11 depicts a strategy for running time-dependent assays in the dark. These assays provide colorimetric outputs, but the fluidic timers provide chemiluminescent signals that are used to indicate the endpoints of the assays. In their current form, the signal from the timer would prompt the operator to take a flash-photograph of the device using a camera-equipped cellular phone (for telemedicine).[5]

Chemiluminescent signals are obtained easily in fluidic timers by pre-depositing and drying the following reagents into the 3D μPAD prior to assembling the device (layers 1–6 are the same as in the device shown in Figure 1): (i) layer 7: 6 μL each of aqueous solutions of luminol (10.7 mM), sodium perborate (45.5 mM), and sodium carbonate (472 mM); and (ii) layer 9: 6 μL of an aqueous solution of potassium ferricyanide (1 M) (Figure S11). The chemiluminescent signal provided by this fluidic timer persists for ca. 10 s, and although the signal is observable easily in the dark, it is not visible in direct light. The chemiluminescent timers must be used within 6 days of preparation: after 6 days of storage (at 23 °C and open to air), the chemiluminescent signal is only faintly visible by eye.

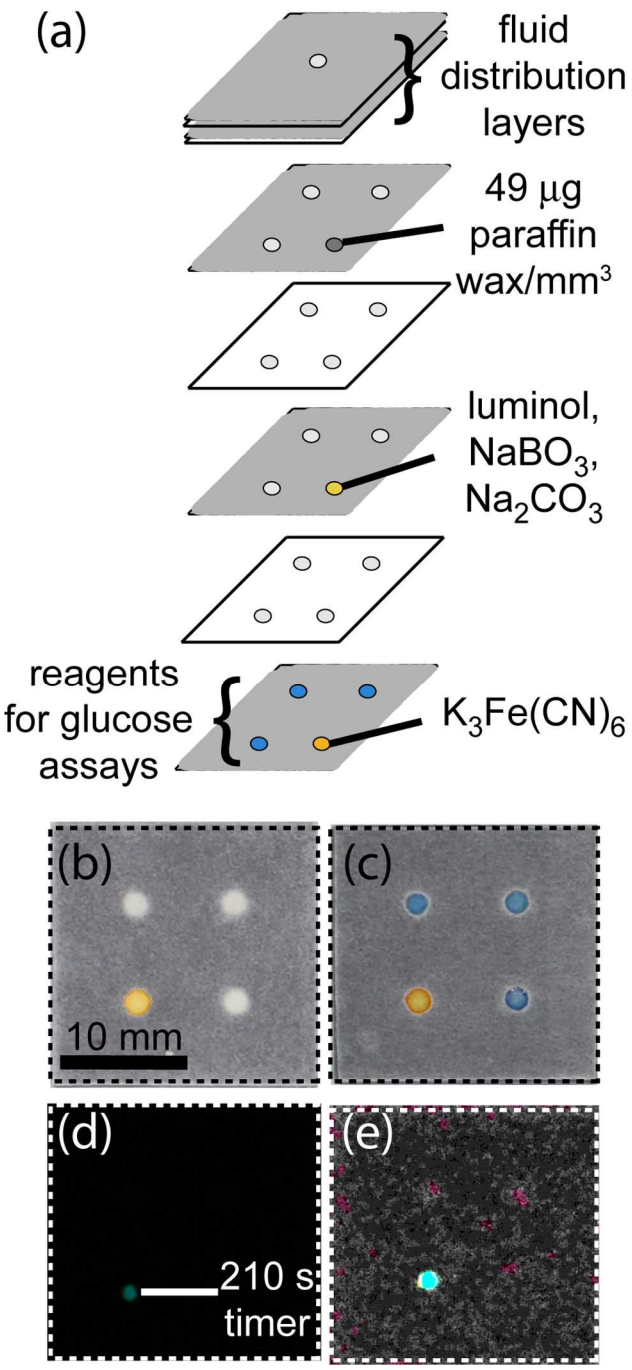


Figure S11. A 3D µPAD containing a fluidic timer that provides a chemiluminescent signal when the assay is complete. (a) Expanded view of the layers of the 3D µPAD. The µPAD has one 210 s fluidic timer, and three conduits for measuring the level of glucose in the sample. (b) Photograph of the bottom of the 3D µPAD prior to applying sample to the device. The

dotted line denotes the edges of the device. (c) Flash photograph of the device 210 s after applying 15 μL of ddH_2O containing 10 mM glucose to the top of the device. (d) Time-lapsed photograph of the bottom of the 3D μPAD 210 s after applying the sample to the top of the device. This photograph was captured in the dark using a 5 s exposure. The blue chemiluminescent signal from the fluidic timer is visible in the bottom left corner of the photograph, and is very apparent by eye (much more so than is obvious from the photograph). (e) Photograph from (d) enhanced with the auto contrast function in Adobe®Photoshop®. The three glucose assays appear as faint red regions, while the μPAD appears predominantly gray.

(c) Audible fluidic timers. Electronic timers are able to create an audible signal, and the ideal fluidic timer would do the same, rather than providing only a colorimetric response. Figure S12 depicts a strategy for incorporating an audible signal into fluidic timers. These assays provide colorimetric outputs, but the fluidic timers provide audible signals that indicate the endpoints of the assays. In their current form, a buzzer sound would prompt the operator to read the assays. The primary advantage of an audible fluidic timer is the ease of conducting an assay (or multiple time-based assays simultaneously) using just a diagnostic device, rather than a diagnostic device paired with an external timer.

To create the audible signals, conductive wires were drawn on the last layer of the fluidic timer using acrylic based silver conductive pens (Figure S12).[7,8] The bottom layer of the device also was equipped with a lithium battery (1.55 V) connected to a piezzo buzzer with an internal drive. Sodium chloride (1.2 μmol) was deposited into the timer observation zone prior to assembling the device (Figure S12a). When this salt is wet by the sample, the resulting conductive solution completes the circuit and triggers the audible alarm.

Figure S12c shows the relationship between time and the intensity of sound pressure produced by the buzzer. The intensity of sound pressure was measured using a sound level meter. The results show that the intensity of sound pressure begins to increase between 200–280 s, and reaches 58 dB (similar to the level of normal conversation) at ca. 230 ± 2.5 s, on average (N = 7). The fluidic timer, however, was designed for 200 s. This apparent decrease in accuracy compared with the colorimetric output indicates the introduction of a new delay in the design of a timer. This new delay time is $T_{solvation}$, which is the time required for the sodium chloride to dissolve and for the solution to reach an ionic strength sufficient for conducting electricity. Future designs of audible fluidic timers will need to account for this time delay as well.

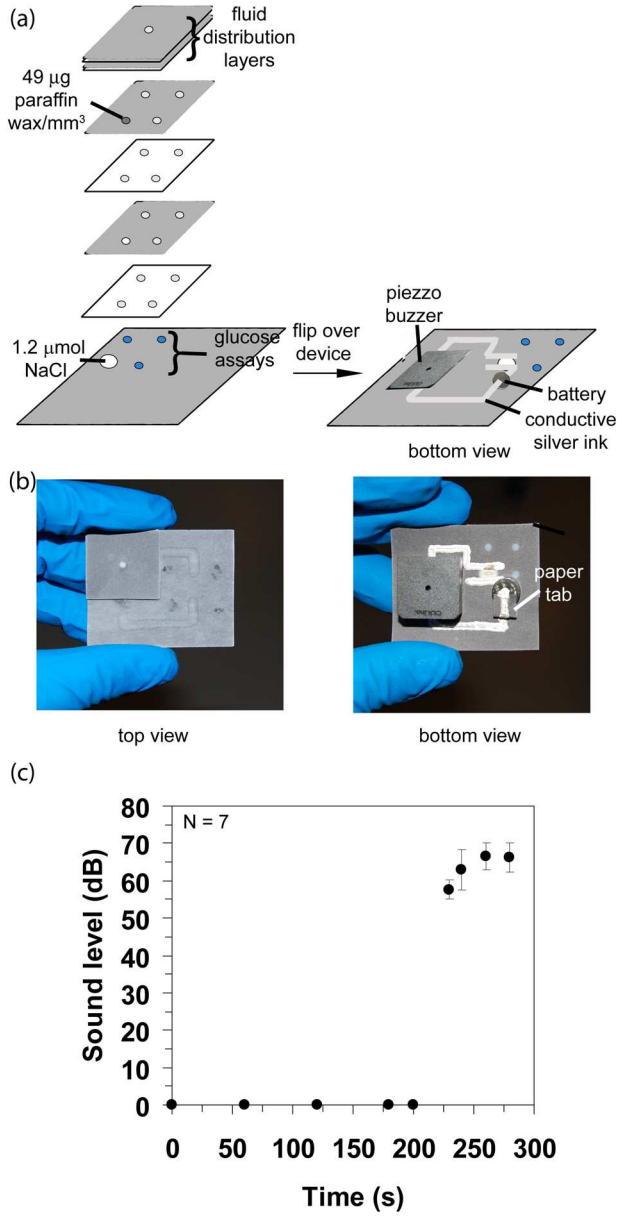


Figure S12. A fluidic timer that provides an audible alarm when the programmed time has elapsed. (a) Expanded view of the layers of the device and the fluidic timer. The device has one 200 s fluidic timer, and three conduits for measuring the level of glucose in the sample. (b) Photographs of the device at t = 280 s. The blue color in the detection zones is the result of the glucose assay. The white lines are conductive wires drawn with a conductive silver pen. A lithium battery and piezzo buzzer with an internal drive were attached to the bottom of the

device with glue prior to assembling the device. (c) Graph showing sound level for the audible timer versus time after adding the sample to the device.

(d) Fluidic timers for lateral-flow assays. As mentioned previously, we view fluidic timers as auxiliary features that could be incorporated into a variety of fiber-based diagnostic devices, including those that move fluids in two dimensions as well as three. Figure S13 depicts one example of a fluidic timer on a 2D device.

When the entry point of the device is dipped into a sample, the sample distributes through the device by capillary action. The sample travels laterally to a diamond-shaped detection zone (where reagents for measuring the level of glucose in the sample were pre-deposited) and to an opposite circular endpoint. At this circular region, the sample wicks in the z-direction through a meter to the bottom of the fluidic timer (see the back view of the device in Figure S13b). The sample then wicks laterally and up through a conduit (containing Yellow 5) to a 2.4-mm diameter circular region on the front of the device. When this region turns from white to orange, the assay is complete. A movie of this process is available in the Supporting Information.

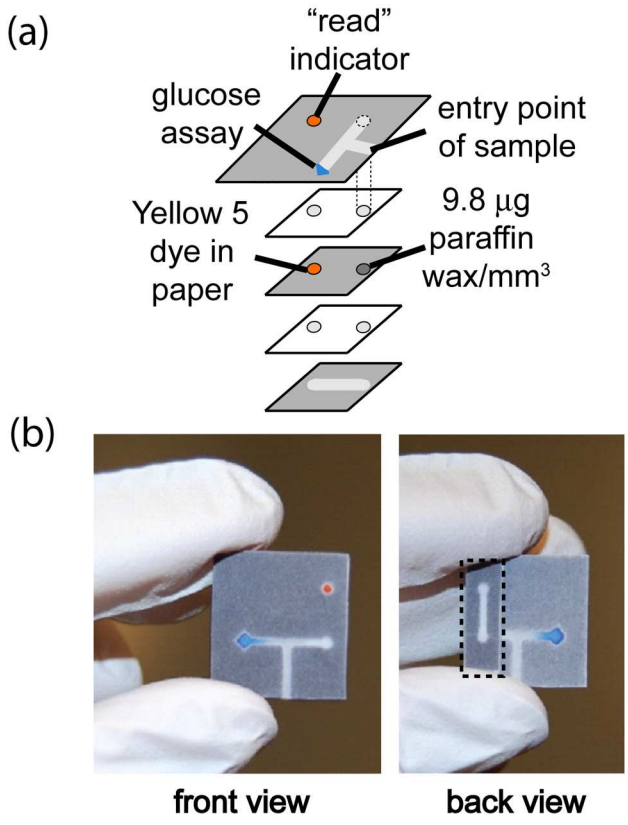


Figure S13. Fluidic timer on a 2D µPAD. (a) Expanded view of the µPAD. Yellow 5 (1 µL) was pre-deposited into the 3rd layer as a 1:5 dilution of food coloring–distilled water prior to assembling the device. The overall dimensions of the 2D µPAD are 25-mm wide × 25-mm long × 0.18-mm thick. The overall dimensions of the fluidic timer are 7.6-mm wide × 24-mm long × ca. 0.9-mm thick. (b) Photographs of the 2D µPAD in (a) after 7 min. The blue color is the indamine dye that is produced when glucose is detected by the mixture of glucose oxidase, horseradish peroxidase, diethyl phenylenediamine, and 1-chloro 4-naphthol (all of which were pre-deposited into the diamond-shaped detection zone prior to assembling the device). The orange color is Yellow 5, which is the colorimetric signal for the timer. The timer is marked by a dotted line on the back view of the 2D µPAD.

Movies showing the distribution of fluids in Figures 1, S10, and S13

The movies are available as separate downloadable files. The movies are accelerated 16× (movie 1) or 32× (movies 2 and 3) the normal speed.

Tables of data

Table S1. Data supporting Figure 2. The time required for distilled water containing 10 mM glucose fluid to complete the glucose assay

	Time (s)	$L_{exp} - L_{paper}$	$(L_{exp} - L_{paper})/L_{max}$[a] × 100	$L_{exp} - L_{paper}$	$(L_{exp} - L_{paper})/L_{max}$[a] × 100
		Assay		9.8 μg paraffin wax per mm^{-3} paper meter:	
5mM glucose solution	5	0	0	0	0
	10	27	30.00	0	0
	20	39	43.33	0	0
	30	60	66.67	0	0
	40	72	80.00	44	34.92
	50	81	90.00	87	69.05
	60	90	100.00	126	100.00
	80	99	110.00	----	----
	110	96	106.67	----	----
	120	79	87.78	----	----
	150	75	83.33	----	----
	170	74	82.22	----	----
	200	71	78.89	----	----
	230	49	54.44	----	----
	260	46	51.11	----	----
1.75mM glucose solution	5	0	0	0	0
	10	2	3.64	0	0
	20	23	41.82	0	0
	30	34	61.82	0	0
	40	36	65.45	30	49.18
	50	44	80.00	58	95.08
	60	55	100.00	61	100.00
	70	55	100.00	----	----
	80	66	120.00	----	----
	100	68	123.64	----	----
	130	55	100.00	----	----
	170	47	85.45	----	----
	200	40	72.72	----	----
	230	30	54.55	----	----

[a] Based on seven measurements.

L_{paper}: background luminosity of the white detection zones, L_{exp}: luminosity of the detection zones during an assay, L_{max}: luminosity obtained at the end of an assay.

Table S2. Data supporting Figure 3c. The time required for aqueous red dye to fill the observation zone in the 5th layer of the μPAD.

Composition of Meter	Turn on time (s) [a]
No wax:	1.11 ± 0.33
10 μg paraffin wax per mm^{-3} paper:	2.33 ± 0.50
19 μg paraffin wax per mm^{-3} paper:	5.11 ± 1.05
29 μg paraffin wax per mm^{-3} paper:	6.44 ± 1.51
39 μg paraffin wax per mm^{-3} paper:	14.33 ± 2.12
49 μg paraffin wax per mm^{-3} paper:	19.22 ± 3.31
53 μg paraffin wax per mm^{-3} paper:	33.56 ± 4.59
54 μg paraffin wax per mm^{-3} paper:	55.78 ± 7.48

[a] Based on seven measurements.

Table S3. Data supporting Figure 4. The time required for aqueous red dye to fill the assay zones and fluidic timer observation zone. Paraffin wax (1.9 μg per mm^{-3} paper) was used to make the timers. The time gaps between assays and timers were calculated by subtraction.

Humidity (%)	Time for assay (s) [a]	Time for timer (s) [a]	Time gap (s) [a]
0	308.57 ± 19.64	485.66 ± 33.62	177.09 ± 19.70
24	273.31 ± 14.30	457.20 ± 19.79	181.89 ± 17.49
37	248.66 ± 16.83	432.77 ± 16.05	184.11 ± 22.40
61	241.46 ± 15.73	435.51 ± 14.07	194.06 ± 20.76
98	241.37 ± 14.53	424.37 ± 27.68	183.00 ± 25.63

[a] Based on seven measurements.

Table S4. Data supporting Figure 5. Effect of paraffin wax per area of paper on contact angle of ddH_2O and gurley porosity.

Wax concentration (μg/mm^3)	Contact angle (°)	Gurley porosity (s/100cm^3) [a]
0	---	2.5 ± 0.1
1.0	97	2.6 ± 0.1
2.0	100	4.2 ± 1.0
9.8	106	15.0 ± 7.3
14.7	111	---
19.6	121	19.0 ± 11.3
29.4	127	43.9 ± 5.7
39.2	130	---
49.0	136	---
52.3	140	---
53.9	140	---
58.8	---	66.8 ± 18.1

[a] Based on eight measurements.

Table S5. Data supporting Figure S12. Time required for the audible alarm to trigger.

Time (s)	Sound level (dB)[a]
0	0
60	0
120	0
180	0
200	0
207	14 ± 24
210	14 ± 25
215	25 ± 24
220	40 ± 27
230	58 ± 3
240	63 ± 5
260	67 ± 4
280	66 ± 4

[a] Based on seven measurements.

Table S6. Data supporting Figure S13. The time required for a solution of glucose (10 mM in distilled water) to complete the glucose assay.

	Time (s)	$L_{exp} - L_{paper}$	$(L_{exp} - L_{paper})/L_{max}$ [a] × 100
Assay	20	0	0
	30	1	1.23
	60	17	20.99
	90	26	32.10
	120	53	65.43
	150	68	83.95
	180	75	92.59
	210	77	95.06
	240	77	95.06
	270	78	96.30
	300	79	97.53
	330	80	98.77
	360	79	97.53
	390	80	100
	420	80	98.77
9.8 μg paraffin wax per mm^{-3} paper meter:	20	0	0
	30	0	0
	60	0	0
	90	0	0
	120	0	0
	150	0	0
	180	0	0
	210	0	0
	240	0	0
	270	0	0
	300	0	0
	330	0	0
	360	5	6.67
	370	23	30.67
	380	44	58.67
	390	61	81.33
	405	68	90.67
	420	75	100

[a] Based on seven measurements.

L_{paper}: background luminosity of the white detection zones, L_{exp}: luminosity of the detection zones during an assay, L_{max}:luminosity obtained at the end of an assay.

Table S7. Data from volunteers for measuring the accuracy and precision of the fluidic timer in Figure 1. The fluidic timer was programmed for 200 s.

Test Subject	Measured Stop Time (s)
1	230
2	200
3	180
4	210
5	190
6	180
7	170
8	190
	Average = 194 ± 19

Table S8. Data supporting Figure 6. Effect of fluid on Contact angles on Whatman chromatography paper that was treated with wax.

Composition of meter	**Contact angle (°)**		
	Water	Bovine serum albumin	Bovine serum
1.0 μg paraffin wax per mm^{-3} paper:	97	93	103
1.5 μg paraffin wax per mm^{-3} paper:	96	92	106
1.9 μg paraffin wax per mm^{-3} paper:	100	96	126
2.4 μg paraffin wax per mm^{-3} paper:	100	95	123
2.9 μg paraffin wax per mm^{-3} paper:	101	104	125
9.8 μg paraffin wax per mm^{-3} paper:	106	108	123
19.6 μg paraffin wax per mm^{-3} paper:	121	113	120
29.4 μg paraffin wax per mm^{-3} paper:	127	117	126

Table S9. Relationship between the type of fluid, the quantity of wax in a timer, and T_{total}.[a,]

[wax] (μg mm^{-3})	Time (s)			
	ddH_2O	10 mM glucose in ddH_2O	200 μM BSA[c] in 0.1 M PBS buffer, pH 7	serum
0	45 ± 4	48 ± 5	69 ± 9	172 ± 15
1.0	64 ± 5	70 ± 8	92 ± 7	203 ± 28
1.3	70 ± 6	70 ± 9	107 ± 14	211 ± 28
1.5	71 ± 3	80 ± 4	138 ± 30	496 ± 223
1.9	84 ± 3	97 ± 7	142 ± 21	>3600
2.9	88 ± 7	98 ± 8	151 ± 20	>3600

[a] N = 8, [c] Bovine serum albumin.

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10. Both of these assays were used simply to demonstrate the concept of including more than one fluidic timer on the same 3D μPAD; the assays do not require different lengths of time to reach completion.