Electronic Supplementary Material (ESI) for Lab on a Chip

A High Throughput Method for Prototyping Three-Dimensional, Paper-Based Microfluidic Devices

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Supporting Information

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Materials

The paper used in these studies was Whatman Chromatography Paper Grade I and the adhesive was 3M™ Super 77™ Multipurpose Adhesive. Erioglaucine, tartrazine, erythrosine, glucose oxidase, horseradish peroxidase, potassium iodide, trehalose, tetrabromophenol blue, anhydrous citric acid, and mono- and di- basic sodium phosphate were purchased commercially and used without further purification. All solutions were prepared using distilled water.

Dye solutions

A 4 mM solution of erioglaucine (blue), a 25 mM solution of tartrazine (yellow), a 12.5 mM solution of erythrosine and a solution of 0.5 mM erioglaucine and 12.5 mM tartrazine (green) were used for imaging the distribution and flow rate of sample in the devices.

General Procedure For Fabricating the Devices:

Patterning Paper

The paper was patterned according to the procedure described references 6 and 19.

Fabricating 3D µPADs

The patterned paper was cut into half sheets (10 cm × 20 cm) and the bottom layer of the design was taped to a sheet of printer paper (21.6 cm × 27.9 cm). The bottom layer of the device was sprayed evenly with adhesive (3M™ Super 77™ Multipurpose Adhesive) for approximately 1 s from a distance of 24 cm. The next layer in the design was aligned with the bottom layer and pressed onto the device. The top of the device was rolled using a rolling pin by applying light pressure. The above steps were repeated with each subsequent layer until all of the layers of the 3D µPAD had been assembled. The final sheet of completed devices was removed from the printer paper, and the sheet was rolled with the rolling pin again using medium pressure before allowing the adhesive to dry for 30 minutes. Individual devices were then cut from the sheet using scissors.

Paper and Tape 3D µPADs

The tape was patterned and the devices were assembled according to the procedures described in reference 6. The dimensions for the paper and tape devices were identical to those used in the corresponding devices prepared using the adhesive method described in this article.

Imaging

All images were acquired using a Nikon D40 digital camera with an AF-S Zoom-Nikkor 18-55mm f/3.5-5.6G ED II lens. Images were analyzed digitally using either Adobe Photoshop or Image J.

Box Design (Fig. 2b)

Adhesive design

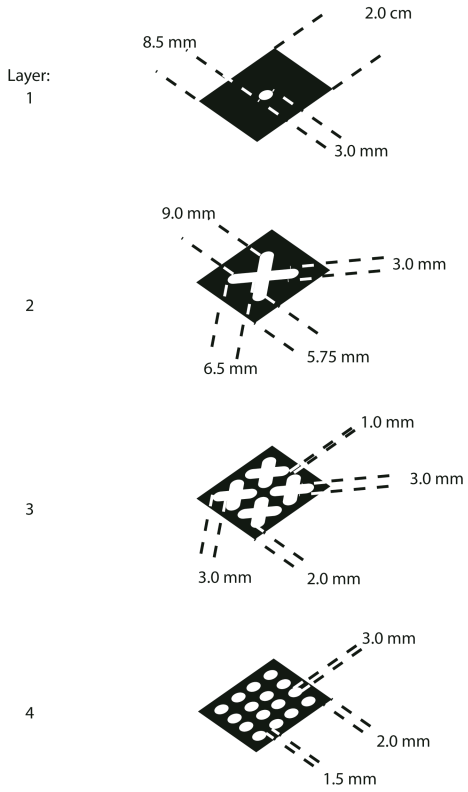

Figure S1. Expanded view of the device shown in Fig. 2b. The device is 20 mm wide × 20 mm long.

Prior to assembling the device, 0.5 µL of each dye solution was deposited into the end of each arm in layer 3. Layer 3 was dried open to the air. After assembly, 40 µL of distilled water was added to the spot on layer 1. The time for all spots to fill with solution was recorded. Layer 4 was imaged after all hydrophilic regions were filled with sample.

Paper and Tape design (for Fig. 2e for comparison with Fig. 2b)

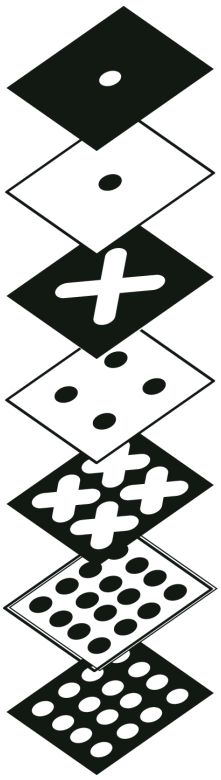

Figure S2. Expanded view of the paper and tape design in Fig. 2e that compliments the device in Fig. 2b. The device is 20 mm wide × 20 mm long.

Rows Design (Fig. 2c)

Adhesive design

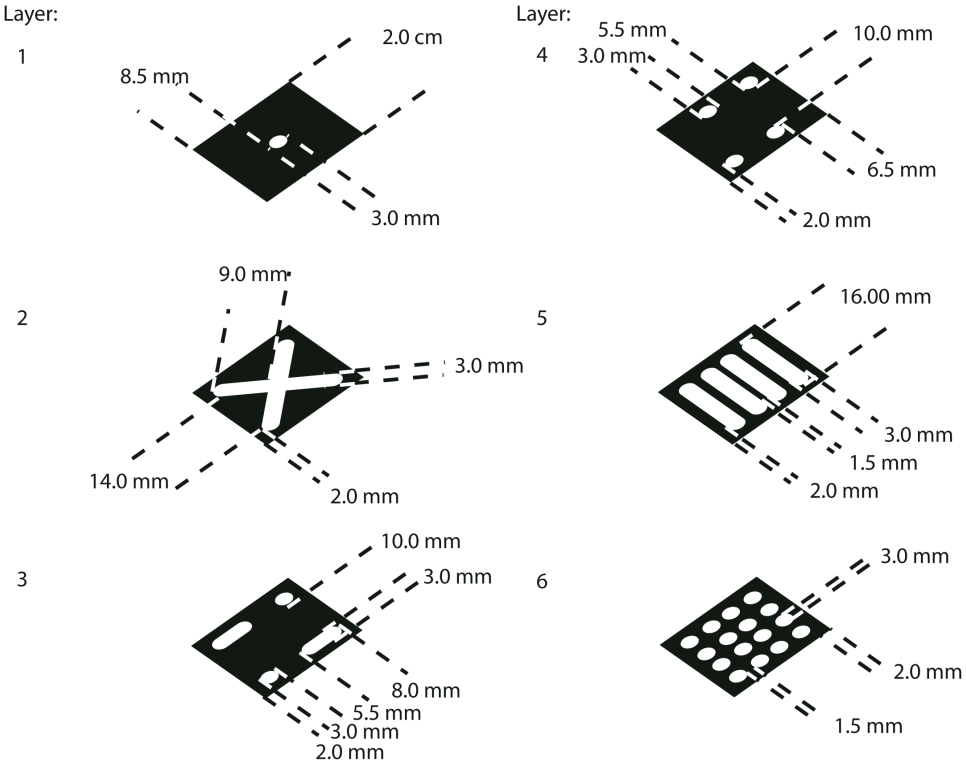

Figure S3. Expanded view of the device shown in Fig. 2c. The device is 20 mm wide × 20 mm long.

Paper and Tape design (for Fig. 2e for comparison with Fig. 2c)

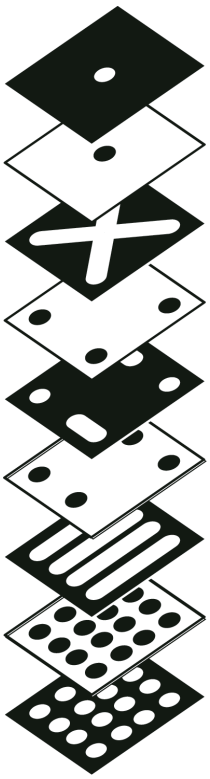

Figure S4. Expanded view of the paper and tape complement to the device in Fig. 2c. The device is 20 mm wide × 20 mm long.

Diagonal Design (Fig. 2d)

Adhesive Design

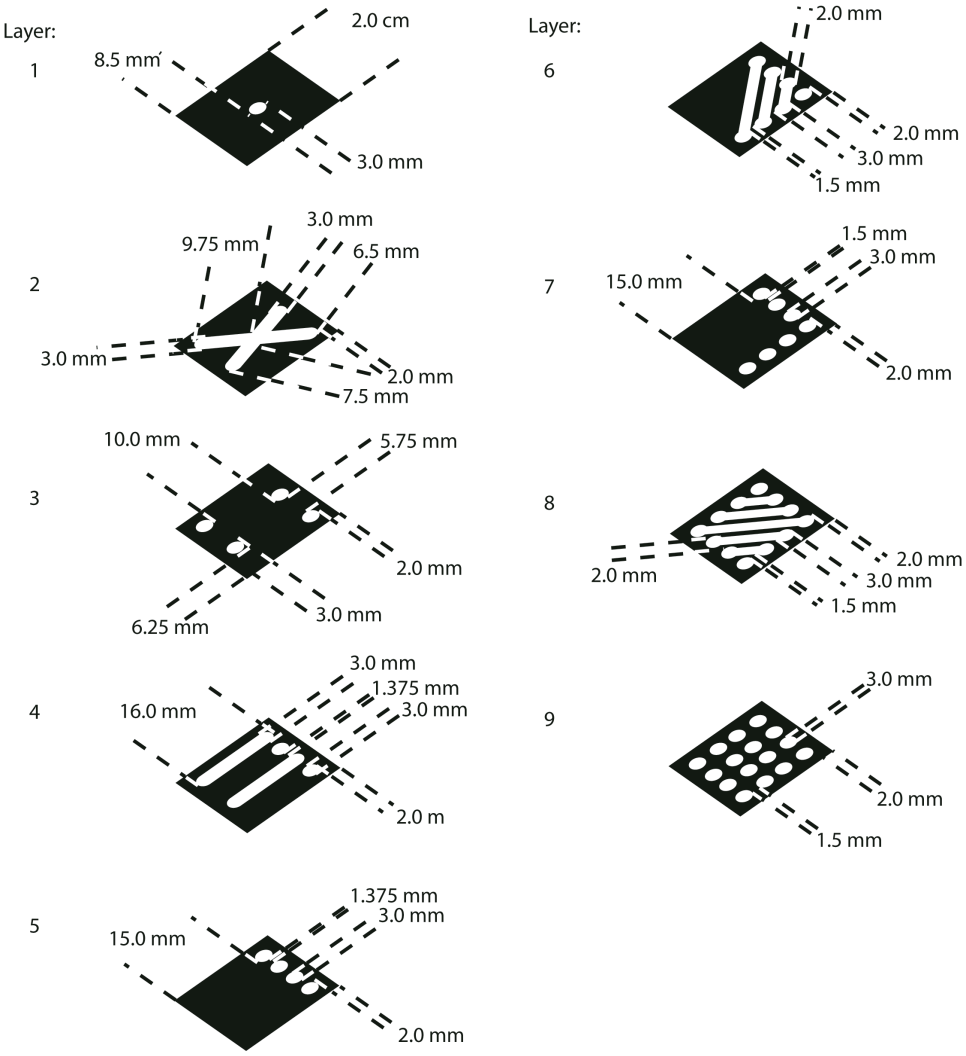

Figure S5. Expanded view of the adhesive diagonal design shown in Fig. 2d. The device is 20 mm wide × 20 mm long.

Paper and Tape design (for Fig. 2e for comparison with Fig. 2d)

Figure S6. Expanded view of the paper and tape device that complements the device in Fig. 2d. The device is 20 mm wide × 20 mm long.

Table S1. Comparison of fill times for devices made using (i) adhesive and (ii) paper and tape. (Note DNF = did not fill in greater than 1.5× the average fill time of identical devices.)

	Paper and Tape			Adhesive		
	Boxes	**Rows**	**Diagonal**	**Boxes**	**Rows**	**Diagonal**
	4:23	18:08	DNF	3:34	5:12	DNF
	4:33	DNF	27:34	1:16	4:28	13:19
	4:53	DNF	27:22	4:31	4:21	19:38
	4:41	18:39	27:31	1:55	DNF	13:58
	4:44	15:55	31:30	1:14	6:20	20:55
	4:54	DNF	30:05	1:52	13:37	16:44
	3:13	10:38	DNF	4:11	6:23	22:07
				DNF		
Average	4:23	15:50	28:48	2:39	6:44	17:47
Standard Deviation	0:33	3:40	1:53	1:23	3:29	3:41

1024-Well Plate Design (Fig. 3)

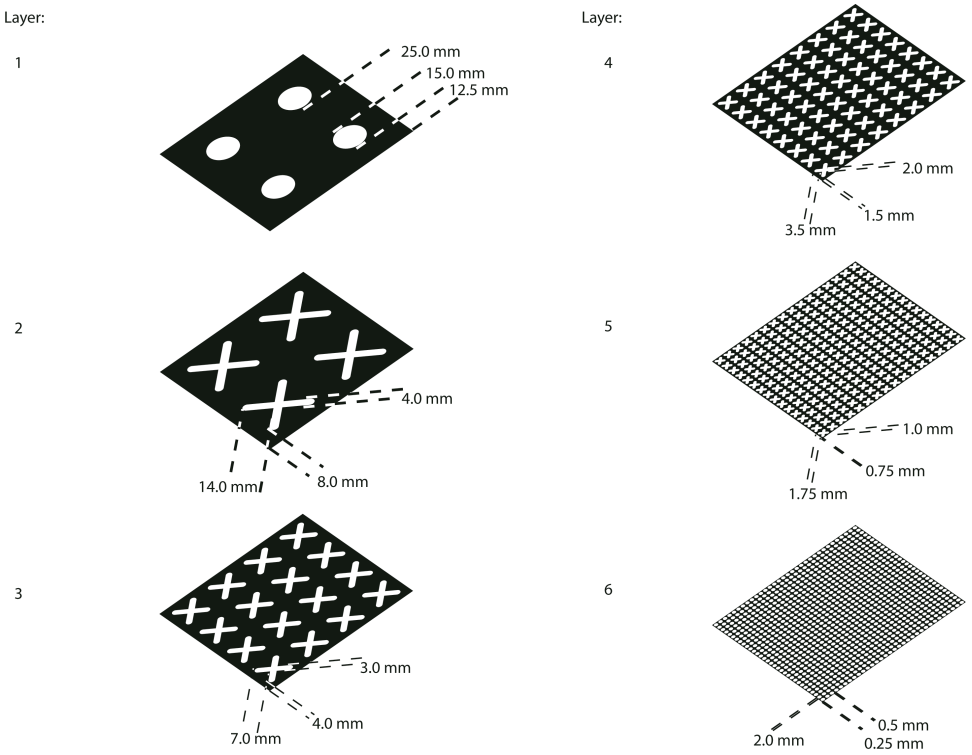

Figure S7. Expanded view of the 1024-well plate design shown in Fig. 3. The device is 80 mm wide × 80 mm long.

The devices were assembled as previously described. Dye solutions (1.0 mL) were added to the device on each spot of layer 1 (different dye on each spot). Layer 6 was imaged after all spots on layer 6 were wet with sample.

Lateral Flow Rate Experiments

Design of the device used to collect the data shown in Fig. 4

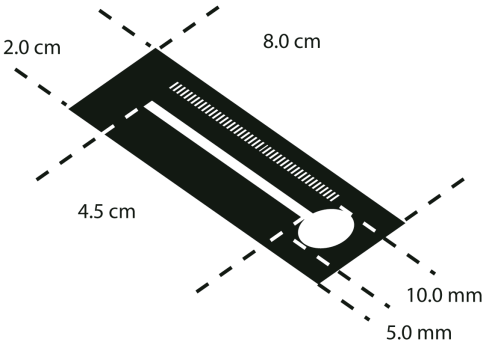

Figure S8. Expanded view of lateral flow device used in Fig. 4. The device is 20 mm wide × 80 mm long.

One side of the device was sealed with tape (Duck® HP260 High Performance Packaging Tape). A group of devices were sprayed with adhesive (on the side opposite the tape). The devices were air dried for 30 minutes prior to use. To all devices were added 100 µL of 1 mM erioglaucine. Photographs were acquired every 10 s over the course of 2 minutes, and then every 30 s until 5 minutes had elapsed. The lateral flow rate was determined using the lines on the device as distance markers; they are spaced 1 mm apart.

Table S2. Data for Fig. 4. Distance (mm) traveled versus time in lateral flow devices. (Note: the average distances were obtained from seven independent experiments using seven lateral flow devices.)

Adhesive			No Adhesive		
Time (s)	**Average Distance (mm)**	**Standard Deviation**	**Time (s)**	**Average Distance (mm)**	**Standard Deviation**
0	0	0	0	0	0
10	4.9	0.7	10	6.0	0.6
20	6.4	0.8	20	7.4	1.0
30	7.7	1.0	30	8.7	1.0
40	8.6	1.1	40	9.9	1.1
50	9.3	1.0	50	10.9	1.3
60	10.3	1.0	60	11.6	1.3
70	11.0	1.2	70	12.6	1.3
80	11.7	1.0	80	13.3	1.4
90	12.3	1.0	90	13.7	1.4
100	12.7	1.0	100	14.4	1.4
110	13.3	1.0	110	15.1	1.6
120	14.3	1.0	120	15.7	1.8
150	15.3	1.0	150	17.3	1.8
180	16.6	1.1	180	18.6	2.0
300	20.4	1.1	300	22.7	2.3

Contact angle measurements

Table S3. Contact angles measurements for comparing wax patterned paper without adhesive with patterned paper that was covered in adhesive. Excess adhesive involved applying 5× the normal quantity of adhesive on the patterned paper.

	No Adhesive	Normal Adhesive	Excess Adhesive
	115.16°	95.92°	85.82°
	108.44°	92.19°	77.64°
	114.36°	98.39°	84.91°
	111.95°	95.13°	81.22°
	108. 81°	93.45°	78.00°
Average	112.48°	95.02°	81.52°
Standard Deviation	3.02°	2.13°	3.80°

Glucose and Protein Assays

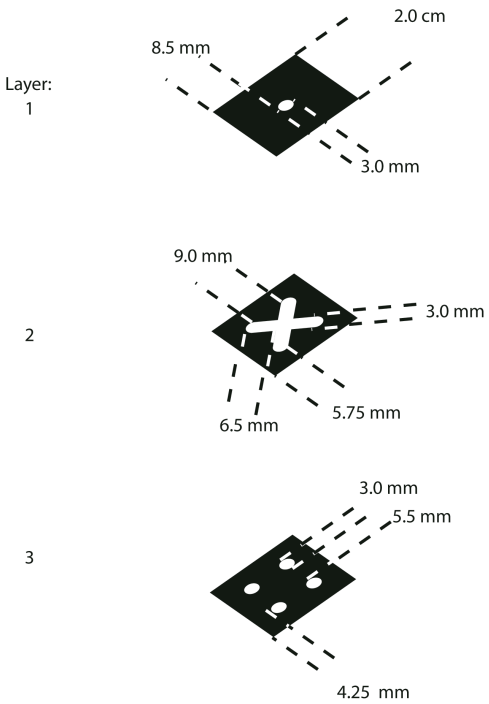

Figure S9. Expanded view of the device used for the glucose and protein assays and for creating the calibration curves shown in Fig. 5. The device is 20 mm wide × 20 mm long.

The protein and glucose assays used identical procedures as described in references 6 and 26. After assembly of the devices, 20 µL of an analyte solution was added to the spot on layer 1. Layer 3 was imaged after a fixed assay time, as described below.

Protein Assay

On layer 3 was spotted 0.2 µL of 250 mM citrate buffer (pH 1.8) containing 8% ethanol (v/v). The solvent was allowed to air dry for 10 minutes. After drying, 0.2 µL of a 9 mM tetrabromophenol blue solution in 95% ethanol (v/v) was spotted in the same location. The assay solution used was bovine serum albumin (BSA) in 200 mM phosphate buffer (pH 6.0), with concentrations that ranged from 0 µM to 50 µM BSA. The assay was run for 5 minutes before layer 3 was imaged.

Glucose Assay

On layer 3 was spotted sequentially 0.2 µL of 0.3 M trehalose in 200 mM phosphate buffer (pH 6.0), 0.2 µL of 0.6 M potassium iodide in 200 mM phosphate buffer (pH 6.0), and 0.2 µL of the enzyme solution [5:1 solution of glucose oxidase: horseradish peroxidase (120 unit of glucose oxidase activity and 30 unit of horseradish peroxidase activity per mL of solution) in 200 mM phosphate buffer (pH 6.0)]. The devices were allowed to air dry for 10 minutes before assembly. The assay solution used was glucose in 200 mM phosphate buffer (pH 6.0), and the concentrations of glucose ranged from 0 mM to 15 mM. The assay was run for 30 minutes before layer 3 was imaged.

Measuring the Concentration of Glucose and Protein

The images were acquired using a Nikon D40 camera and were analyzed using either Image J or Adobe Photoshop. The glucose assays were quantified using Image J. Split channel colors was applied to each image and then the mean intensity of each reaction spot was determined using the histogram function. The average intensity for each concentration was taken. The difference of the intensities from assays that contained 0 mM glucose were used to create the calibration curve in Fig. 5. The protein assays were quantified using Adobe Photoshop. The protein assay images were converted to CMYK mode and the mean cyan intensity of each reaction spot was determined. The average intensity for each concentration was taken. The difference of the experimental intensities from assays for 0 µM BSA were used to create the calibration curve in Fig. 5.

Table S4. Data used to create the calibration curve in Fig. 5 for the protein assay. (Note: for all concentrations of protein, including the control, the intensities of nine assay spots were measured.)

Mean Intensity of Cyan in CMYK Mode from Protein Assay							
	25 µM BSA	**17.5 µM BSA**	**12.5 µM BSA**	**7.5 µM BSA**	**5 µM BSA**	**2.5 µM BSA**	**0 µM BSA**
	131.00	151.91	172.68	197.78	183.87	216.82	228.80
	147.02	152.90	170.51	203.39	186.78	218.18	229.09
	145.24	160.02	173.11	194.15	185.50	217.35	228.30
	139.68	149.34	170.92	199.52	178.23	201.00	216.35
	143.33	150.47	170.17	194.74	180.53	203.80	222.38
	142.25	151.81	170.33	195.36	178.74	194.46	213.54
	140.92	158.00	167.91	199.23	177.29	170.21	211.31
	145.84	165.14	176.50	205.84	181.75	179.22	218.90
	137.44	147.48	166.07	199.76	175.71	167.73	209.50
Average	141.41	154.12	170.91	198.86	180.93	196.53	219.80
Standard Deviation	4.97	5.74	3.01	3.92	3.83	20.04	7.716

Table S5. Data used to create the calibration curve in Fig. 5 for the glucose assay. (Note: for all concentrations of glucose, including the control, the intensities of nine assay spots were measured.)

Mean Intensity of Blue Channel from Glucose Assay							
	0 mM Glucose	**1 mM Glucose**	**2.5 mM Glucose**	**5 mM Glucose**	**10 mM Glucose**	**15 mM Glucose**	**20 mM Glucose**
	84.372	80.261	73.238	55.167	40.535	13.918	14.918
	83.09	77.498	65.389	43.442	38.683	6.921	7.134
	85.252	75.468	68.173	45.118	28.29	27.194	16.209
	89.998	79.222	68.214	47.228	35.085	28.482	20.16
	94.303	75.39	72.575	47.966	32.108	24.027	42.341
	91.756	82.538	70.297	61.81	31.377	24.605	10.067
	95.291	89.393	80.392	68.004	28.058	33.047	42.438
	94.287	80.481	82.368	58.144	40.271	37.677	16.972
	92.427	85.442	73.811	60.687	48.915	30.231	26.466
Average	90.086	80.632	72.717	54.174	35.925	25.122	21.856
Standard Deviation	4.687	4.605	5.635	8.608	6.825	9.489	12.879