A continuous-ink, multiplexed pen-plotter approach for low-cost, high-throughput fabrication of paper-based microfluidics

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Supplementary Information

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Methods

Materials

Hardware setup: A desktop pen plotter, (AxiDraw) from Evil Mad Scientist Laboratories, CA, US; Double-ended Comix marker from Comix Group Co. Ltd., Shenzhen, China, where the fine and broad tip ends used are 0.5 and 2.0 mm in diameter, respectively; Chromatography paper (Whatman No. 1) from GE healthcare life sciences, IL, US; Objet 30 Prime 3D printer from Stratasys Ltd., MN, US; Ink reservoir (DIY CISS Continuous Ink Supply System) from BCH Technologies, NC, US; Technical drawing pen (1.2 mm) from Shanghai Hero Plotter instrument Co., Shanghai, China; Cold-laminating layer (self-adhesive laminate sheets) from Fellowes, IL, US; Hot-laminating layer (GBC EZUse thermal laminating Pouches) from Swingline, IL, US; Benchtop laminator (GBC Inspire) from Swingline, IL, US; Super hydrophobic solution from NeverWet LLC, PA, US; Aniline dye stain (Oil soluble) from J.E. Moser's, WY, US.

Reagents for biological assays: Sulfanilamide (S9251), N-(1-naphthyl) ethylenediamine dihydrochloride (33461), Citric acid (251275), Sodium nitrite (237213), 4-diethylaminobenzaldehyde (156477), Tetrabromophenol blue (199311), Bovine Serum Albumin (BSA) (A7906), Cumene hydroperoxide (247502), 3, 3', 5, 5'-tetramethylbenzidine (TMB) (860336), Methyl red sodium salt (114502), Bromothymol blue (114413), Sodium hydroxide (S8045), Hydrochloric acid (258148), Potassium phosphate buffer, pH 6 (01476), Buffer solution pH 9 (456101) all from Sigma- Aldrich, MO, US; Phosphate buffer saline (10010023) from Thermo Fisher Scientific Inc., MA, US; Urobilinogen (sc-296690) from Santa Cruz Biotechnology, TX, US.

Design and 3D printing of the multipen holder

To increase the throughput of patterning for fabrication of paper-based microfluidics, we custom-designed a low-cost multipen holder and printed it using a polyjet 3D printer. The 3D-printed multipen holder is able to hold 8 pens to plot 8 patterns at the same time. To investigate the accuracy of the Axidraw pen plotter integrated with the multi-pen holder, circles with diameters of 4 mm were plotted by both fine and broad tips of the Comix marker at different plotting speeds. Images of the patterns were captured and analyzed using a MATLAB script (MathWorks, MA, US) to measure the deflection of each pen as well as the deflection of the whole plotting system at different plotting speeds in both the X- and Y-directions. Moreover, we investigated the roundness and uniformity of the plotted circles by measuring their inner and outer radii. We used a MATLAB script and measured the inner and outer radius of each circle at 8 different points (at angles of 0, 45, 90, 135, 180, 225, 270 and 315 degrees) and reported the average and standard deviation of the radii for each circle.

Lamination of the paper

The fabricated paper-based microfluidic device is comprised of chromatography paper patterned with hydrophobic resin and backed with a laminating layer to seal the device and improve its mechanical strength. To investigate the effectiveness of the laminating layer to seal the back of the chromatography paper, two different lamination methods have been tested: cold- and hot-lamination. Cold-laminating and hot-laminating layers were aligned on the back of the chromatography paper and laminated with a benchtop laminator in cold and hot modes, respectively. The performance of the plotted patterns on both hot- and cold-laminated papers was studied in two different cases: plotting patterns before and after the lamination. A matrix of circles with a diameter of 4 mm was prepared with SolidWorks 2014 (Dassault Systèmes SolidWorks Corp., France) and plotted by the AxiDraw pen plotter on both hot- and cold-laminated papers. Different plotting speeds ranging from 1% to 110% were tested with varying repetitions (1, 3, and 5 passes over the same pattern). The water resistance of the patterns on the laminated paper was investigated by spotting a dyed aqueous solution in the center of each circle. Moreover, to investigate the mechanical strength of the laminated papers, tensile tests were performed based on ASTM D638 standard for a type-IV specimen. The hot- and cold-laminated papers were cut and tested by a universal testing machine (eXpert 3910, ADMET, MA, US). Furthermore, to study the mechanical strength of the laminated paper after loading an aqueous sample, a tensile test was performed on patterns that had been loaded with 20 µl of dyed aqueous solution at the center of circle.

Continuous ink supply

To improve the limited ink capacity of the commercial permanent markers, a continuous ink supply system was developed and connected to technical pens. The ink reservoir was connected to the cartridge of a technical pen by tubing. The air release hole of the technical pen was blocked to seal the technical pen reservoir from the atmosphere and thereby generate negative pressure in the technical pen reservoir as the ink is depleted while plotting (**Figure 4a**). This negative pressure in the technical pen chamber causes a flow of ink from the main ink reservoir to the technical pen reservoir. We prepared a hydrophobic ink by dissolving an oil-soluble dye in super-hydrophobic solution. The components of NeverWet hydrophobic ink are given in the **Table S-1**. The performance of the prepared hydrophobic ink in the continuous ink system was then studied by patterning the chromatography paper with different plotting speeds and investigating the water resistance of the resulting patterns.

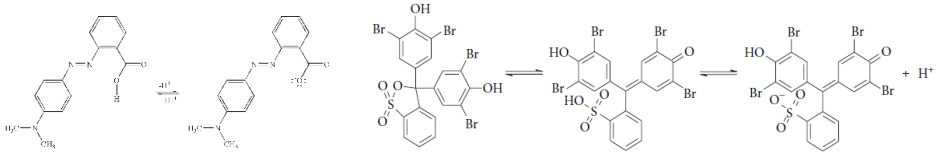
Table S-1: The components of the hydrophobic ink

Chemical Name	Weight %
Mineral Spirits	<90.0
Aromatic Petroleum Distillates	<10.0
Naphthalene	<1.0
Aniline Dye Stain	<1.0

Colorimetric biological assays

To demonstrate the applicability of the proposed fabrication method for paper-based microfluidics and to verify their effectiveness, we conducted five colorimetric assays[1-3]: nitrite, urobilinogen, protein, blood, and pH (**Table S-2**). 0.5 µl of the reagent solution was spotted in the detection zone and left to dry at room temperature for 10 minutes. Each sensor was then validated by applying 3 µL of an artificial urine sample, (engineered with varying concentrations of each analyte in a physiologically relevant range) and measuring the color the change at each concentration.

Table S-2: Colorimetric reagents, color change reaction, and physiologically-relevant analyte range

Nitrite assay [1]	
Reagents	50 mM sulfanilamide, 10 mM N-(1-naphthyl) ethylenediamine dihydrochloride, 330 mM citric acid
Reaction	Sulfanilamide + NO_2^- $\xrightarrow{acid}$ Diazonium salt Diazonium salt + N-(1-naphthyl)ethylenediamine → Magenta azo dye
Analyte range	0.078, 0.156, 0.312, 0.625, 1.25, and 2.5 mM
Urobilinogen assay [3]	
Reagents	0.1 M 4-(Dimethylamine)benzaldehyde
Reaction	Urobilinogen + 4-(dimethylamine)benzaldehyde $\xrightarrow{acid}$ Pink azo dye
Analyte range	0.2, 1, 2, 4, 8, and 12 mg/dL
Blood assay	
Reagents	6.6% w/w cumene hydroperoxide, 4.0% w/w 3, 3', 5, 5'-tetramethylbenzidine
Reaction	cumene hydroperoxide + 3,3',5,5'-tetramethylbenzidine $\xrightarrow{hemoglobin}$ 3,3',5,5'-tetramethylbenzidine diimine (blue)
Analyte range	2.5, 5, 10, 25, 50, 80, 100, 200 red blood cells/µL
Protein assay[2]	
Reagents	3.3 mM tetrabromophenol blue, 250 mM citric acid
Reaction	Based on the protein error of indicators, where protein alters the color of acid-base indicators without changing the pH of the solution by accepting hydrogen ions from the indicator
Analyte range	15, 30, 100, 300, 2000 and 2500 mg/dL bovine serum albumin
pH assay [3]	
Reagents	0.02% methyl red sodium salt 0.25% of bromothymol blue
Reaction	 Yellow Red || Yellow Green Blue
Analyte range	pH 5, 6, 7, 8, and 9

Supplementary Figures

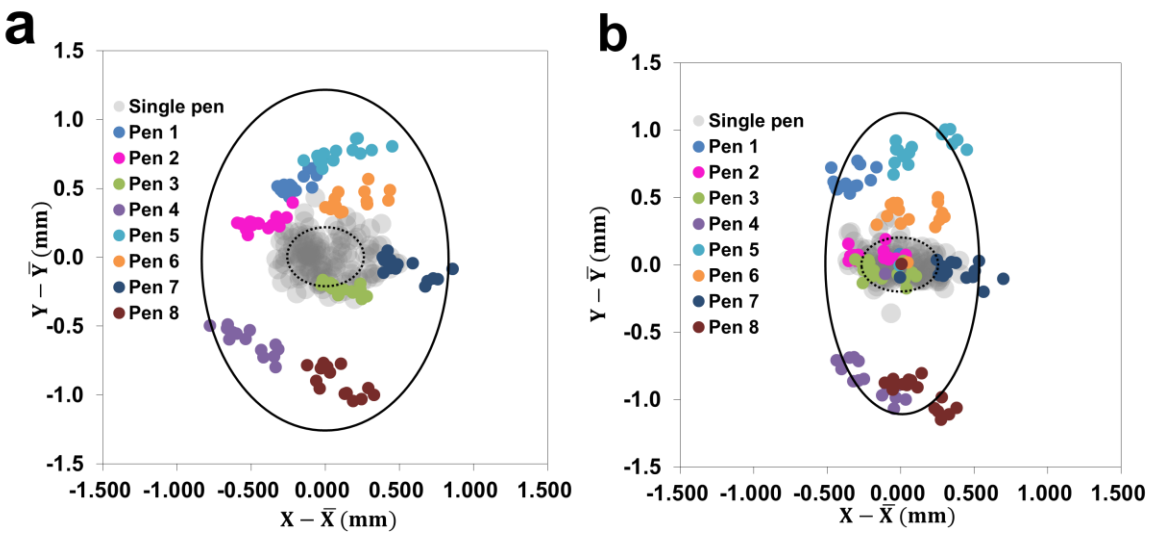


Figure S-1. Accuracy of patterns drawn by using eight pens simultaneously vs. a single pen. (a) Deflections of the plotted patterns with the fine tip by using eight pens simultaneously vs. a single pen. **(b)** Deflections of the plotted patterns with the broad tip by using eight pens simultaneously vs. a single pen. In this figure, $\overline{X}$ and $\overline{Y}$ are the mean deflections in the X- and Y-directions. The solid and dotted lines represent the 95% confidence ellipses for eight pens and a single pen plotting, respectively.

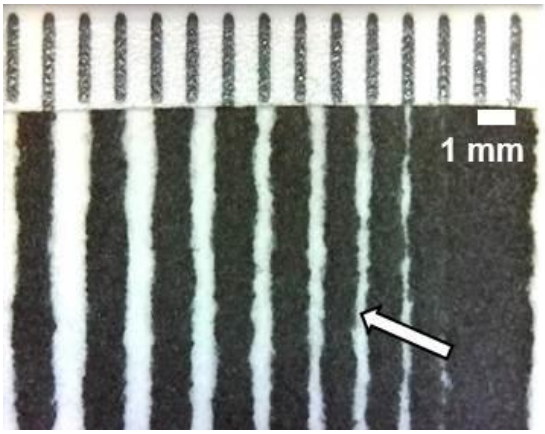


Figure S-2. Pattern used for measuring the resolution of barriers and channels. The resolution of the barriers and channels are 1023±118 μm and 391±68 μm, respectively (indicated by the white arrow).

Supplementary Table

Table S-3. The mean and standard deviation of the deflections of the patterns plotted by using eight pens simultaneously vs. a single pen. All units are in millimeters.

	Fine tip				Broad tip			
	$\bar{X}$	$\bar{Y}$	σ_X	σ_Y	$\bar{X}$	$\bar{Y}$	σ_X	σ_Y
Multipen	0.4013	-0.5573	0.4011	0.6103	0.405	-0.5413	0.2252	0.5541
Single-pen	-0.02	0.14	0.182	0.1414	0.06	-0.43	0.195	0.1363

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