Supporting Information

Wax Printing – a Simple Micropatterning Process for Paper-based Microfluidics

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Experimental Procedures

Preparation of Paper Before Patterning. Starting from sheets of Whatman No. 1 Chr chromatography (460 mm × 570 mm), we cut each sheet into four US Letter size sheets (215 mm × 280 mm). This paper size fits directly into the manual feed tray from the printer. Any other size of paper can be also be used, but requires some adjustment of the printer settings.

Designing and Printing of the Devices. We used a drawing software CleWin® (PhoeniX Software, The Netherlands) that was designed for designing circuit boards. Given the low resolution of the process, any drawing software could be used instead. CleWin generates PostScript files, which were converted into a PDF file for printing. The final image preserves the designed dimensions with good accuracy (< 10% variation of the intended feature size). We used the default printer settings for photo-quality printing.

General Preparation of the Devices. Placing the printed paper in a hot plate set at 150 °C for two minutes reflowed of the ink on the paper. To achieve best results, we flip the paper over a couple of times after one minute over the hot plate. Most devices in this article provided investigatory function and were ready for use after the melting step without addition of any chemicals. Multi-zone paper plates such as shown in Figure 4A and 4B were also ready for use right off the hotplate. For functional μPADs such as the example shown in Figure 4C it was necessary to add chemicals to the test zones before using the device.

Measuring the Limiting Features of the Devices. We tested many design of the devices in different shapes, orientations, line thickness, and line spacing to allow easy and simple visualization if the features, *i.e.*, lines, gaps, and circles, could function as barriers, channels, and reservoirs, respectively. Analysis of the effectiveness of the hydrophobic barriers and hydrophilic channels were visual; we used a solution of 5 mM of Amaranth [CAS number 915-67-3]. The presence of leaks indicated that the barrier was not effective at a given line thickness; blockage of the solution

was an indication that the spacing between two lines were too close to leave a channel for wicking of the solution. To probe for a functional barrier or reservoir, we added an excess of volume of a colored solution and monitored that there was no leak over 30 min, time that was long enough to evaporate the solution. To probe for a functional channel, we observed that solution wicked from one reservoir into another over a minimal distance of 5 mm.

We measured all line thickness and distances using an optical microscope (Leica MZ12) and a 1-mm scale, or using the ruler tool from Adobe Acrobat on images acquired with a desktop scanner (Epson Perfection) with resolution of 300 dpi or greater.

Preparation of Devices for Bioassays. Reagents for a protein assay, a cholesterol assay and a glucose assay were added to each test zone as follows:

Protein Assay. A priming solution (0.2 μL, 250-mM citrate buffer, pH 1.8, prepared in 92% water and 8% ethanol by volume) was spotted in the protein test zone using a micro-pipette (VWR) and was allowed to dry for 10 min at ambient temperature. A reagent solution (0.2 μL, 9-mM tetrabromophenol blue prepared in 95% ethanol and 5% water by volume) was spotted on top of the priming solution and dried for 10 min under ambient conditions.

Cholesterol Assay. A reagent solution [cholesterol oxidase-horseradish peroxidase (200 units of cholesterol oxidase enzyme activity and 30 units of horseradish peroxidase enzyme activity per mL of solution), 0.6-M potassium iodide, and 0.3-M trehalose in a pH 7.0 phosphate buffer prepared in Millipore-purified water] was spotted in the cholesterol test zone using a micro-pipette and allowed to dry under ambient conditions.

Glucose Assay. A reagent solution [glucose oxidase-horseradish peroxidase (120 units of glucose oxidase enzyme activity and 30 units of horseradish peroxidase enzyme activity per mL of solution), 0.6-M potassium iodide, and 0.3-M trehalose in a pH 6.0 phosphate buffer prepared in

Millipore-purified water] was spotted in the glucose test zone using a micro-pipette and allowed to dry under ambient conditions.

Performing bBoassays. A negative control solution (phosphate buffer saline, pH 7.4), and a positive control solution (15-μM bovine serum albumin (BSA), 40-mM cholesterol, and 5-mM glucose prepared in PBS, pH 7.4) were prepared, and 5 μL of each sample was transferred to a Petri dish using a micro-pipette. The bottom of the device was dipped into each solution (~5 μL), and the device wicked the solution into the test zones. After remaining upright in the Petri dish for 30 min, the devices were scanned using an Epson Perfection 1640SU scanner on default settings (color photo, 600 dpi).

Additional Results. Figure SI-1 shows all measurements for vertical and horizontal lines as barriers and as gaps in function of the nominal width, i.e., the value defined in the drawing program. The data from these experiments yielded the functions to predict the final dimensions of lines and gaps in a μPAD.

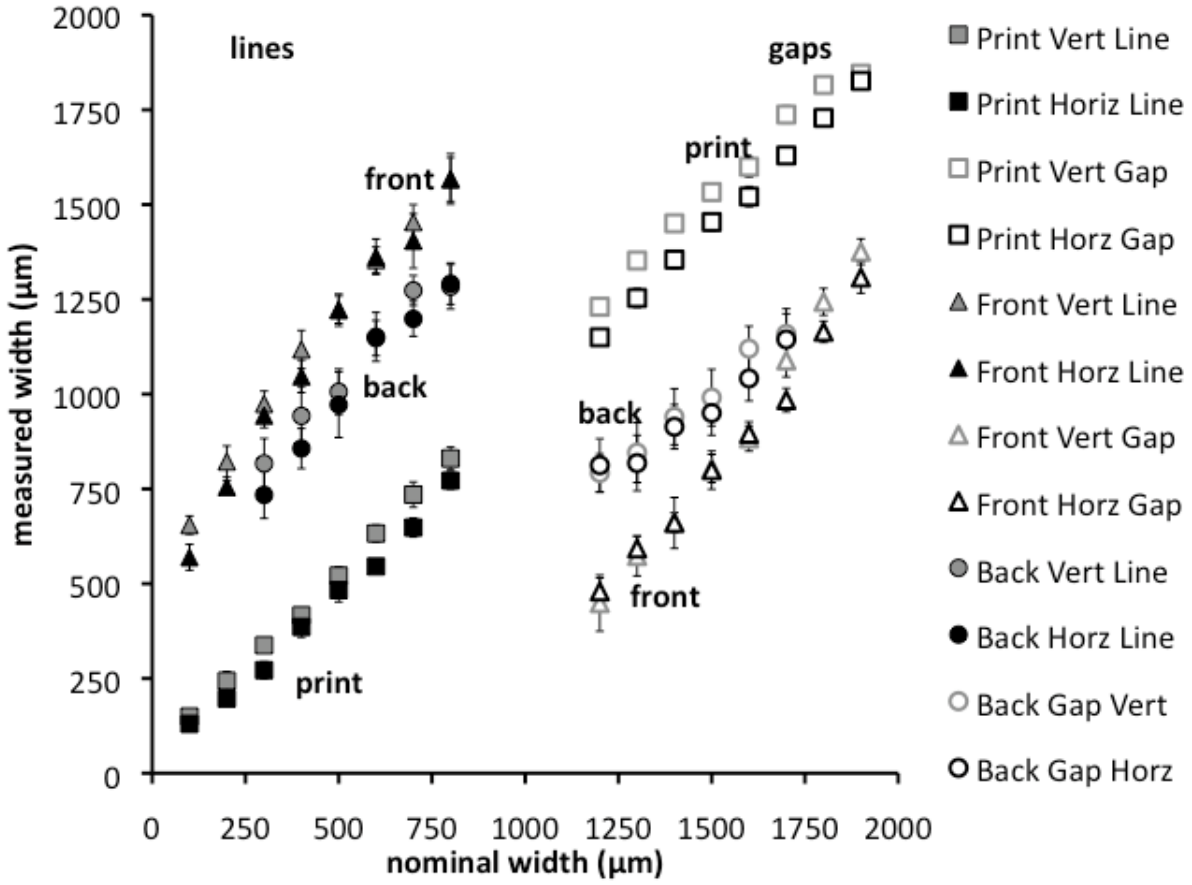

Figure SI-1. Measurements of line (barrier) and gaps (channel) widths before (print) and after (front and back) the melting process as a function of the nominal widths defined in the drawing program. The error bars represent one standard deviation (n = 10).