

Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics

Emanuel Carrilho,^{*,†,‡} Andres W. Martinez,[†] and George M. Whitesides^{*,†}

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, and Instituto de Química de São Carlos, Universidade de São Paulo, 13566-590 São Carlos-SP, Brazil

This technical note describes a detailed study on wax printing, a simple and inexpensive method for fabricating microfluidic devices in paper using a commercially available printer and hot plate. The printer prints patterns of solid wax on the surface of the paper, and the hot plate melts the wax so that it penetrates the full thickness of the paper. This process creates complete hydrophobic barriers in paper that define hydrophilic channels, fluid reservoirs, and reaction zones. The design of each device was based on a simple equation that accounts for the spreading of molten wax in paper.

This technical note describes a simple method, which we term wax printing, for fabricating microfluidic paper-based analytical devices (μ PADs) by patterning hydrophobic walls of wax in hydrophilic paper using a commercially available printer and hot plate. The fabrication process involves two core operations: (i) printing patterns of wax on the surface of paper and (ii) melting the wax into the paper to form complete hydrophobic barriers (Figure 1). Wax printing is rapid, inexpensive, and particularly well-suited for producing large lots (hundreds to thousands) of prototype μ PADs. This work was carried out independently of a similar and complementary study by Lu et al.,¹ in which waxed-based printing was also used to fabricate μ PADs. Herein, we describe the procedure for patterning paper by wax printing, we derive a simple model to account for the spreading of molten wax in paper that facilitates the design of μ PADs, we define the resolution of the fabrication method, and we demonstrate four examples of μ PADs fabricated by wax printing: (i) a modified 96-zone paper plate with microfluidic channels for sample distribution; (ii) the equivalent of a 384-zone microtiter plate made in patterned paper; (iii) a device for detecting glucose, cholesterol, and proteins; and (iv) a three-dimensional μ PAD made by stacking layers of patterned paper and double-sided adhesive tape.

We and others are developing μ PADs as platforms for diagnostic devices and for other applications in analysis.^{2,3} Micro-

PADs are typically small, portable, and fabricated from inexpensive materials. Because they can operate without any supporting equipment, we believe they will be well-suited for diagnostic applications in developing countries, in the field by first responders, or in home healthcare settings. Thus far, μ PADs have been fabricated by photolithography,^{2,4,5} plotting,⁶ plasma oxidation,⁷ cutting,⁸ and inkjet printing.⁹ Each method has its own set of advantages and limitations, but wax printing involves the fewest number of steps and is best suited for fabricating large numbers (>100) of μ PADs in a single batch. With wax printing, we can pattern approximately 100–200 μ PADs on an 8.5 in. \times 11 in. sheet of paper with a single print and heat cycle in <5 min. Micro-PADs can be fabricated for \sim \\$0.001/cm² using Whatman chromatography paper (\sim \\$7/m²). The cost of the solid ink is \sim \\$0.0001/cm² of paper assuming 20% coverage of ink. In other words, the material cost of the device depends almost entirely on the type of paper that is used and can be \\$0.001 or less for devices made from inexpensive papers like paper towels (\sim \\$0.2/m²).²

The features made using wax printing are not as highly resolved as those generated by photolithography,² but they are adequate for most applications of μ PADs. When the wax on the surface of the paper melts, it spreads vertically as well as laterally into the paper. The vertical spreading creates the hydrophobic barrier across the thickness of the paper. The lateral spreading decreases the resolution of the printed pattern and results in hydrophobic barriers that are wider than the original printed patterns. Paper is usually anisotropic, the fibers tend to be more horizontal than vertical, and as a consequence, lateral spreading of fluids in paper is usually more rapid than vertical spreading. This effect produces hydrophobic barriers that are wider on the front face of the page, where the line was printed, compared to the back face of the page. The spreading of molten wax in paper complicates the design of μ PADs since the dimensions of the printed features on paper do not translate directly to the dimensions of hydrophobic patterns in paper. We studied the spreading of molten wax in paper and derived an expression that relates

* To whom correspondence should be addressed. E-mail: gwhitesides@gmwhgroup.harvard.edu (G.M.W.); emanuel@iqsc.usp.br (E.C.).

[†] Harvard University.

[‡] Universidade de São Paulo.

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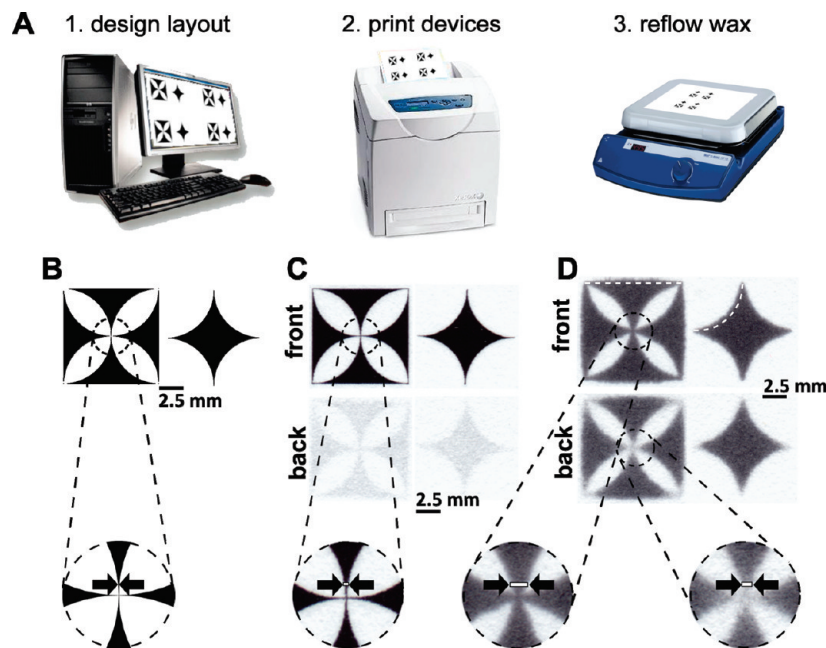


Figure 1. Patterning hydrophobic barriers in paper by wax printing. (A) Schematic representation of the basic steps (1–3) required for wax printing. (B) Digital image of a test design. The central area of the design was magnified to show the smaller features. (C) Images of the test design printed on Whatman no. 1 chromatography paper using the solid ink printer. The front and back faces of the paper were imaged using a desktop scanner. (D) Images of the test design after heating the paper. The dashed white lines indicate the original edge of the ink. The white bars in the insets highlight the width of the pattern at the position indicated by the arrows.

the width of a hydrophobic barrier to the width of the original printed line. This relationship helps to calculate the dimensions of a printed pattern required to produce a given μ PAD.

Model to Account for the Spreading of Molten Wax in Paper. The spreading of molten wax in paper is a process of capillary flow in porous materials that is described by Washburn's equation (eq 1):¹⁰

$$L = (\gamma Dt / 4\eta)^{1/2} \quad (1)$$

where L is the distance that a liquid of viscosity η and surface tension γ penetrates a porous material with an average pore diameter D in time t . The viscosity of the wax is a function of the temperature, and a uniform and well-controlled heat source is required for reproducible results. Assuming the paper is kept at a constant temperature throughout the heating step, all of the parameters in eq 1 are fixed in our experiments and the distance that the wax will spread in the paper from the edge of the printed line will be constant, regardless of the width of the printed line, so long as the amount of wax is not limiting, which is the case for thin lines. The width of the hydrophobic barrier is thus related to the width of the printed line by eq 2:

$$W_B = W_P + 2L \quad (2)$$

where W_B is the width of the barrier, W_P is the width of the printed line, and L is the distance that the wax spreads from the edge of the printed line (all given in micrometers), in a direction perpendicular to the line (Figure 2A). The value of L can be determined experimentally by measuring the width of printed lines and the width of the resulting hydrophobic barriers.

To calculate the width of a hydrophilic channel defined by two parallel hydrophobic barriers, we use eq 3:

$$W_C = W_G - 2L \quad (3)$$

where W_C is the width of the hydrophilic channel and W_G is the space between the two printed lines (also in micrometers), measured on the edge of the line.

EXPERIMENTAL DESIGN

Choice of Paper. We used Whatman no. 1 chromatography paper in most of this study because it is hydrophilic, homogeneous, pure, reproducible, biocompatible, and available. It is also relatively inexpensive, costing approximately \$7/m². It is available in sheets of 460 mm \times 570 mm, which fits four U.S. Letter sheets, 215 mm \times 280 mm, that can be fed directly into the printer. We also tested the method with regular print paper and TechniCloth.

Choice of Printer and Heat Source. We used a Xerox Phaser 8560N color printer because it is designed to print a wax-based ink. The print head dispenses ink (melted wax) as liquid droplets of approximately 50–60 μ m in diameter on the surface of the paper, where they cool and solidify instantaneously without further spreading. The ink is made of a mixture of hydrophobic carbamates, hydrocarbons, and dyes that melts around 120 $^{\circ}$ C and is then suitable for piezoelectric printing.¹¹

We used a digital hot plate because it provides a flat, uniformly heated surface for heating the paper. Other heat sources, such as ovens or heat guns, can also be used for wax patterning.

Measuring the Spreading of Molten Wax in Paper. We designed a series of lines of varying widths (100–800 μ m, in

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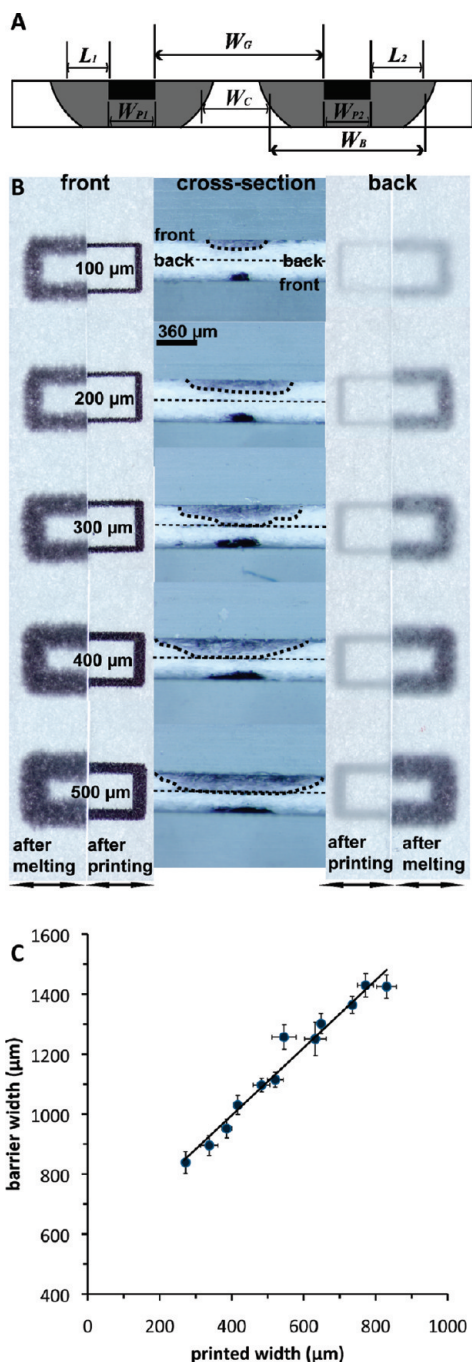


Figure 2. Spreading of wax in paper to form hydrophobic barriers. (A) Schematic representation of the spreading of molten wax in paper and definition of the variables for rational design of μPADs : W_P is the printed width of the line, W_G is the separation (or gap) between the edge of the lines before melting, W_B is the thickness of the hydrophobic barrier defined as the middle point between the front and back widths (average width), W_C is the width of the resulting channel after melting of the wax, also defined at the average between the front and back values, and L is the spreading of the wax in relation to the original edge of the line. The black rectangles represent the wax before the heating step, and the gray area represents the wax after the heating step. (B) Optical micrographs comparing the front, back, and cross-sectional view of printed horizontal lines "before" and "after" the melting process. The lines had nominal widths of 100–500 μm . (C) Quantitative assessment of the spreading of molten wax in chromatography paper. The values represent the average ($n = 10$) of the measured barrier widths, and a linear fit yielded $W_B = 1.1W_P + 550$, $R^2 = 0.97$. The error bars represent 1 standard deviation in both axes ($n = 10$).

increments of 100 μm), printed them, heated them to melt the wax into the paper, and then analyzed the cross sections of the resulting hydrophobic barriers. For each line, we determined the nominal width (the width of the line as designed on the computer), the printed width (the width of the line as printed on paper), and the barrier width (the average of the width of the hydrophobic barrier on the front face of the paper and back face of the paper).

Resolution of the Wax Printing Method. To define the resolution of the wax printing method, we determined experimentally the barrier width of the narrowest functional hydrophobic barrier and the channel width of the narrowest functional hydrophilic channel. We defined a functional hydrophobic barrier as one that prevented water from wicking across it for at least 30 min. We defined a functional hydrophilic channel as one that was at least 5 mm long and wicked aqueous solutions from a fluid reservoir to a test zone.

To determine the narrowest functional hydrophobic barrier, we fabricated a series of test barriers with nominal widths ranging from 100 to 600 μm , in increments of 100 μm . We tested horizontal straight lines (i.e., parallel to the arrangement of the printing nozzles), vertical straight lines (i.e., perpendicular to the arrangement of the printing nozzles), and circles. To determine the narrowest functional hydrophilic channel, we fabricated a series of channels defined by two parallel lines with nominal widths of 400 μm . The nominal space between the two lines was varied from 400 μm to 1.1 mm, in increments of 100 μm .

EXPERIMENTAL DETAILS

Patterning Paper by Wax Printing. We designed patterns of hydrophobic barriers as black lines on a white background using drawing software (Clewint). The patterns were printed on Whatman no. 1 chromatography paper using the solid ink printer set to the default parameters for photoquality printing. The printer can print an 8.5 in. \times 11 in. sheet of paper in approximately 5 s. The printed paper was then placed on a digital hot plate set at 150 $^\circ\text{C}$ for 120 s, and the wax melted and spread through the thickness of the paper (Figure 1). The patterned paper was ready for use after removing the paper from the hot plate and allowing it to cool to room temperature (<10 s). A complete description of the experimental procedures is included in the Supporting Information.

RESULTS AND DISCUSSION

Model to Account for the Spreading of Molten Wax in Paper. The average spreading of wax in paper (L) was found to be 275 μm for lines with nominal widths ≥ 300 μm (Figure 2C). We observed that the measured printed width can differ from the nominal width by as much as 10% of the nominal width and that the average printed width was ~ 30 μm larger than the nominal width for vertical lines and ~ 25 μm smaller than the nominal width for horizontal lines. This difference indicated a bias in the orientation of printing. Hydrophobic barriers from lines with nominal widths less than 300 μm did not contain enough wax to span the entire thickness of the paper, and we did not consider them in our model. Figure 2B compares side-to-side lines of 100–500 μm before and after the melting process. Analysis of the cross-section of these lines provided the insight for the proposed

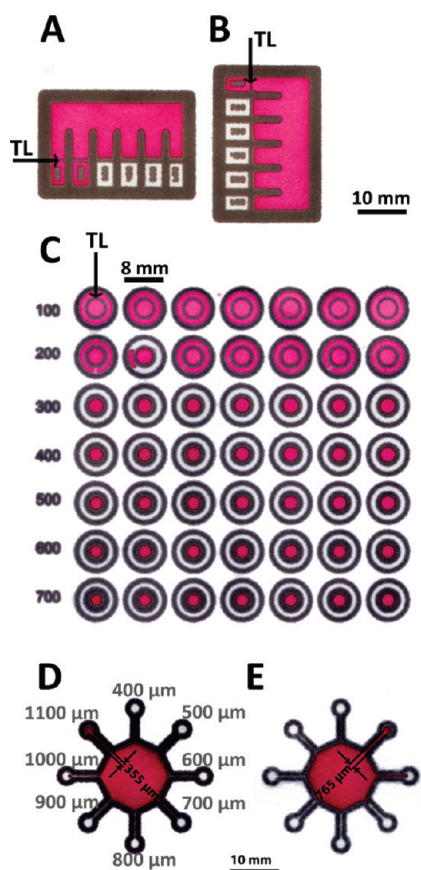


Figure 3. Resolution of the printing method. The smallest functional horizontal (A), vertical (B), and circular (C) barriers were determined by testing barriers with a range of nominal widths (100–700 μm , in 100 μm increments). (A and B) For vertical and horizontal hydrophobic barriers, we fabricated a device with a central fluid reservoir and six test zones. Each test zone was separated from the fluid reservoir by a hydrophobic barrier indicated as the test line (TL). The features inside the test zones are the nominal widths of the barriers (in micrometers), but they were blurred during the heating step. (C) For circular hydrophobic barriers, we designed a circular fluid reservoir separated from a concentric circular test zone by the hydrophobic barrier. (D) The smallest functional hydrophilic channel was determined by testing channels with a range of gap widths (400–1100 μm , in 100 μm increments) defined by hydrophobic barriers of constant nominal width (400 μm). The channels separated a central fluid reservoir from the test zones. (E) Image of the back face of the device shown in part D. The values shown in gray are the nominal gap widths. The numbers shown in black are the average channel widths ($n = 12$) measured after the heating process.

model for the spreading of molten wax in paper. Figure 2C shows that the width of the barrier is linearly dependent on the printed width (W_p) as predicted by eq 2. The data shown here was derived from a larger data set shown in Figure SI-1 in the Supporting Information.

Resolution of the Wax Printing Method. The smallest functional hydrophobic barriers had nominal widths of 300 μm , which resulted in an average barrier width of 850 ± 50 μm ($n = 10$). These results agree well with our model, which predicts a barrier width of 850 μm . As it was expected based on the results of the spreading of wax in paper, barriers with nominal widths ≥ 300 μm generated functional hydrophobic barriers in 100% of the experiments, regardless of the orientation of the line ($n = 7$ for horizontal and vertical lines, $n = 75$ for circles) (Figure 3A–C).

The 200 μm wide test line showed some differences in the results for the horizontal, vertical, and circular lines that confirms the bias in the orientation of printing: the 200 μm wide vertical test lines generated functional barriers in 86% of the experiments ($n = 7$), while the 200 μm wide horizontal and circular test lines generated functional barriers in only 14% of the experiments ($n = 7$ for horizontal lines, $n = 75$ for circles). Since printed circles are effectively a combination of vertical and horizontal lines, it makes sense that the circles and horizontal lines yielded identical results. Finally, none of the 100 μm -wide test lines yielded functional hydrophobic barriers ($n = 7$ for straight and vertical lines, $n = 75$ for circles). It should be noted that these results only apply to Whatman grade 1 Chr paper (180 μm thick) and will be different for other papers.

The smallest functional hydrophilic channel had an average width of 561 ± 45 μm ($n = 12$) and came from two printed lines separated by a nominal width of 1100 μm (Figure 3D,E). These results also agree well with our model described in eq 3, which predicts a channel width of 550 μm for two lines separated by 1100 μm .

The resolution of wax printing is coarse, i.e., the boundaries between hydrophilic and hydrophobic regions on the paper are not sharp. The root-mean-square (rms) roughness at the edge of a 300 μm line, after melting, was approximately 57 μm . The resolution is limited by the quality of the paper (thickness, porosity, and orientation of fibers). It is however, possible to improve the mass transport of the wax in the perpendicular direction (through the plane of the paper) by applying an external force, such as vacuum driven flow of air, on the direction of the flow (results not shown). Additionally, printing a pattern on both sides of the paper could lead to smaller and more highly resolved barriers but would require careful alignment of the patterns.

Solvent Compatibility. Wax-printed μPADs are compatible with aqueous solutions. Aqueous solutions of various pHs, acids (sulfuric acid, 30%, and hydrochloric acid, 1 N), bases (sodium hydroxide, 0.1 N), and glycerol (pure or in solution) wick along the hydrophilic channels but do not cross the hydrophobic barriers, even with a large excess of fluid. Strong acid and base solutions dissolve the paper if the device is left in the solution for long periods of time (days). Wax-printed channels are not compatible with organic solvents. We tested xylenes, acetone, methylene chloride, mineral oil, and alcohols (methanol, ethanol, and *n*-propanol), and they all wicked through the hydrophobic barriers. Dichloromethane and acetone wash away most of the dye in the wax and carry it along with the front of the solvent, but after the solvent evaporates, the hydrophobic barriers are still present. This permeability to organic solvents may lead to new experimental opportunities and assays. For example, we could apply biological samples on a 96-zone paper plate and, after the samples are dry, wash the samples with an organic solvent to remove endogenous and exogenous interferences for a given bioassay.

Micro-PADs Made by Wax Printing. We fabricated four examples of μPADs with different designs and functions to demonstrate that wax printing is capable of generating paper-based multizone plates,¹² lateral-flow devices,⁴ and three-dimensional

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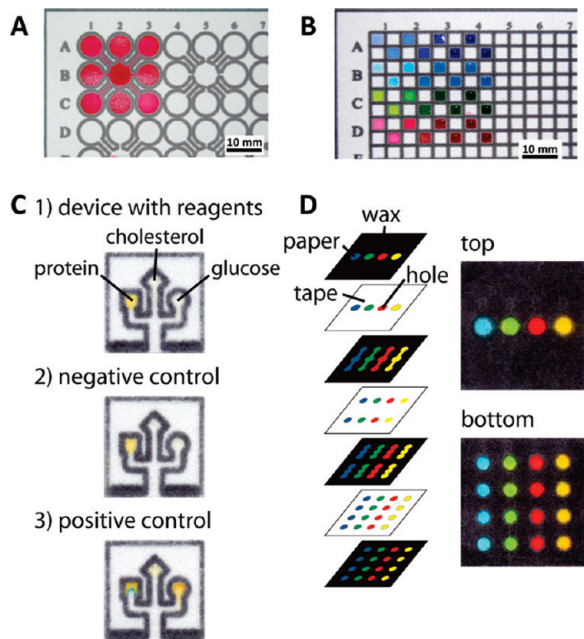


Figure 4. Examples of wax-printed μ PADs. (A) An example of a 96-zone plate with microfluidic channels for sample distribution. We applied 45 μ L of a solution of Amaranth in water in the central zone; the liquid distributed itself homogeneously into all eight surrounding zones. (B) An example of a 384-zone paper plate after application of 1–8 μ L of several dyes. (C) An example of a μ PAD for detecting total protein, cholesterol, and glucose in biological fluids. The reagents for each assay were added to each test zone before the device was used. The negative control wicked a phosphate buffer saline solution (PBS), while the positive control wicked a solution containing 15 μ M bovine serum albumin (BSA), 40 mM cholesterol, and 5 mM glucose in PBS. (D) Demonstration that wax printing is suitable for fabrication of 3D μ PADs by stacking layers of patterned paper and double-sided adhesive tape. The device distributes four individual samples (we show aqueous dyes) from inlets on the top of the device into an array of 16 test zones on the bottom of the device. The schematic diagram illustrates the layers of patterned paper and tape in the device.

(3D) μ PADs¹³ (Figure 4). Parts A and B of Figure 4 show paper-based multizone plates (e.g., 96- and 384-zone paper plates) that are compatible with plate readers for quantitative analysis in both absorbance and fluorescence modes.¹² Fabrication of multizone paper plates requires only printing lines thick enough to hold a large excess of liquid within the zones, 500 μ m in these examples. Wax printing takes less than 3 min to prepare four multizone plates, while photolithographic methods require about 20 min to prepare a single plate.¹² We fabricated a simple lateral-flow μ PAD for colorimetric detection of protein, cholesterol, and glucose in biological fluids (Figure 4C).^{2,4} The device has a central inlet channel that wicks a sample from the bottom of the device and distributes it into three independent test zones that are prespotted

with reagents for the assays. To demonstrate that wax printing is compatible with previously published methods for fabricating 3D μ PADs,¹³ we fabricated a simple device for sample distribution by stacking layers of patterned paper and double-sided adhesive tape (Figure 4D).

CONCLUSIONS

Wax printing is a rapid, efficient, and inexpensive method for fabricating μ PADs. Devices can be prototyped by wax printing in less than 5 min (from design to finished prototype). After testing the prototype, small changes in the design, for example to adjust the channel width, can be realized within minutes. The cost of the materials to fabricate a full 8.5 in. \times 11 in. sheet of μ PADs is \sim \\$0.60 for Whatman no. 1 chromatography paper. The design of the hydrophobic barriers and hydrophilic channels made by wax printing is complicated by the spreading of molten wax in paper, but we introduce two simple equations that predict the final width of hydrophobic barriers and channels and facilitate the design process.

We foresee wax printing as an ideal method for large-scale production of μ PADs. Wax printing could be adapted to a reel-to-reel process, where rolls of paper would first go through a wax printer, then through an oven for the heating process, and finally, through an inkjet printer where reagents for assays or other applications could be printed in the test zones. Current wax printing technology can be transferred immediately to laboratories without capability for photolithography, and since the know-how necessary for development of analytical devices based on paper is minimal, we hope this work will encourage other scientists to propose new applications for μ PADs.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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