Transfer Wax Printing Characterization: Expanding Wax Printing Capabilities to Various Paper-Types

by
Anthony To

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Bioengineering
(Honors Scholar)

Presented November 28, 2016
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Anthony To for the degree of Honors Baccalaureate of Science in Bioengineering presented on November 28, 2016. Title: Wax Transfer Printing Characterization: Expanding Wax Print Capabilities to Additional Paper-Types.

Abstract approved: ____

Elain Fu

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Key Words: Wax printing, paper microfluidics, transfer patterning, intermediate template

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Anthony To, Author

Wax Transfer Printing Characterization: Expanding Wax Printing Capabilities to Additional Paper-Types

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Abstract: Currently, wax printing is limited to materials that can be processed by a wax printer. Wax transfer printing is a method that allows previously unusable materials to be patterned with wax via an intermediate template. Millipore cellulose, a material too thick to be processed by a wax printer, was patterned using this process and the resulting wax patterns characterized. Overall, the method has potential for use in paper microfluidics as a simple, inexpensive, and rapid process that expands the range of materials that can be patterned with wax.

1. INTRODUCTION

Paper-based microfluidics is a research field that utilizes paper as the main fabrication substrate and patterned microstructures are used to generate complex microfluidic functions[1]. Its aim is to develop simple, inexpensive, portable, and easy-to-use devices for point-of-care scenarios in low-resource settings[1,2]. In paper-based microfluidics, wax printing has been commonly used for the fabrication of devices. Wax printing is a relatively simple, versatile, and inexpensive fabrication process, where wax is placed onto a paper's surface by a printer. It is then baked to allow the wax to penetrate the paper to create hydrophobic barriers that can retain fluid and manipulate flow[1]. Wax printing currently utilizes a computer drawing software to design a wax pattern, a wax printer to print the wax pattern, and a heat source to melt the wax pattern into the paper to create the hydrophobic barriers[1,3].

Wax printing has limitations. For example, wax printing only works with paper substrates that can be mechanically processed by the wax printer. Papers that are too fragile, thick, coarse, large, or small are not compatible for processing by the printer. Their use can damage the substrate itself, or the printer through printer jams or ink jet nozzle scarring. The wax printer used in this study has an area density range between 60 g/m^2 to 220 g/m^2 and only accepts paper dimensions between 76 mm by 127 mm to 216 mm by 610 mm. Thus, wax printing is limited to substrates that satisfy these criteria.

A 2011 microfluidic study "*Patterned Paper as a Low-Cost, Flexible Substrate for Rapid Prototyping of PDMS Microdevices via 'Liquid Molding'*" by Yao Lu and others, introduced a modified wax printing process called "*wax transfer printing*"[4]. This process uses an intermediate template that can be mechanically processed by the wax printer, but unlike paper, this template does not absorb the wax during the baking process. Instead, the wax remains on the template surface and is subsequently transferred onto a paper substrate by placing the template into contact with the paper substrate and applying heat. This patterns wax onto the desired paper substrate.

The template used in the 2011 study was a transparency film[4]. Transparency films are composed of plastic and do not absorb wax. Since wax printers can process transparency films, the desired wax pattern can be printed onto the transparency film and then transferred onto another surface under proper transfer conditions. The 2011 study further described using glass plates and clamps to sandwich the wax printed transparency film to the target paper in order to help facilitate the transfer process[4].

While the 2011 study suggested a novel solution to a major limitation in wax printing, main drawbacks of the study were the lack of characterization of the wax transfer process and the limitation to nitrocellulose for demonstration of the method. This led to our motivation to create a working protocol for wax transfer printing for a material of interest to our lab that cannot be directly wax printed

In this study, Whatman no.1 filter paper was the initial paper used to characterize wax transfer printing because it is an inexpensive paper that has been widely used in wax printing. Using this paper, we compared "direct" wax printing to wax transfer printing parameters. Once these essential parameters were determined, the techniques were then applied to a material of interest. Millipore cellulose was selected for this study because its microfluidic properties differ from Whatman no.1, such as a larger capacity. However, with an area density of 290 g/m^2, it is not recommended for processing with the wax printer.

2. MATERIALS & METHODS

Materials & Equipment.

The wax patterns were designed using the computer drawing software Draftsight (Dassault Systèmes, France). The wax printer used was a Xerox printer (8580/DN, ColorQube, CN) with its settings set to photo-quality mode in order to print with the highest wax output. The baking equipment was an oven from Thermo Scientific (659, Precision Model, Gaithersburg, MD). The transparency film (8.5" by 11", OHP Film, Pictorico) used as the intermediate template was selected because it had a special coating that resisted warping and melting at high temperature. Whatman no.1 filter paper (1001917, 47 cm by 56 cm, Whatman) and Millipore Cellulose (CFSP22300, EMD Millipore, Darmstadt, Germany) were used as the target papers for the transfer wax printing process. The Whatman no.1 filter paper came as a 47 cm by 56 cm sheet that was trimmed to a standard 8.5 inches by 11 inches' paper sheet using a CO_2 laser cutting system (H-Series, Full Spectrum Laster, Las Vegas, NV) so that it can be processed by our wax printer. Aluminum foil (01-213-100, Fisherbrand™, Fisher Scientific) was used as a barrier to prevent the transparency film from bonding to the glass plates. The glass plates used were borosilicate glass plates (229 mm by 254 mm, MatterHackers, Orange County, CA) which has thermal expansion and shock resistance. Four c-clamps (HD-2016-0301, 1" clamps, Husky) were used to compress and hold the glass plates, transparency film, aluminum foil, and target paper together. Images were captured using a desktop scanner at 300 dpi or at 1600 dpi (Perfection V700 photo scanner, Nagano, Japan). The wax patterns were measured using the computer measurement program ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA,).

Wax Transfer Printing Method

The transfer process can be divided into three major steps: (1) wax printing onto the transparency film, (2) assembling the sandwich system, (3) baking for wax penetration into the porous substrate. A schematic of the entire transfer process can be seen in Figure 1. The design used in this study was composed of eight rectangular reservoirs with nominal dimensions of 1 cm by 4 cm. The reservoir nominal line width was varied between 600 µm to 2000 µm in order to control the amount of wax entering the system. These reservoirs were wax-printed onto a transparency film to initiate the transfer process. It should be noted that the transparency film used in this study had a coated side and a non-coated side. The non-coated side was selected for the wax transfer process because the coating resulted in the transparency film bonding to the target paper. The transparency film with the wax-printed reservoirs was placed onto the target paper. Aluminum foil was added on top of the transparency film's coated side to prevent the transparency from sticking to the glass plates, which in turn helped aid the transfer process (Supporting Information). These components were then placed between glass plates and compressed using c-clamps. This sandwich system was then baked in the oven at 150°C for five minutes. The wax melting point is around 120°C, so selecting a higher temperature reduced the total baking time. After the first

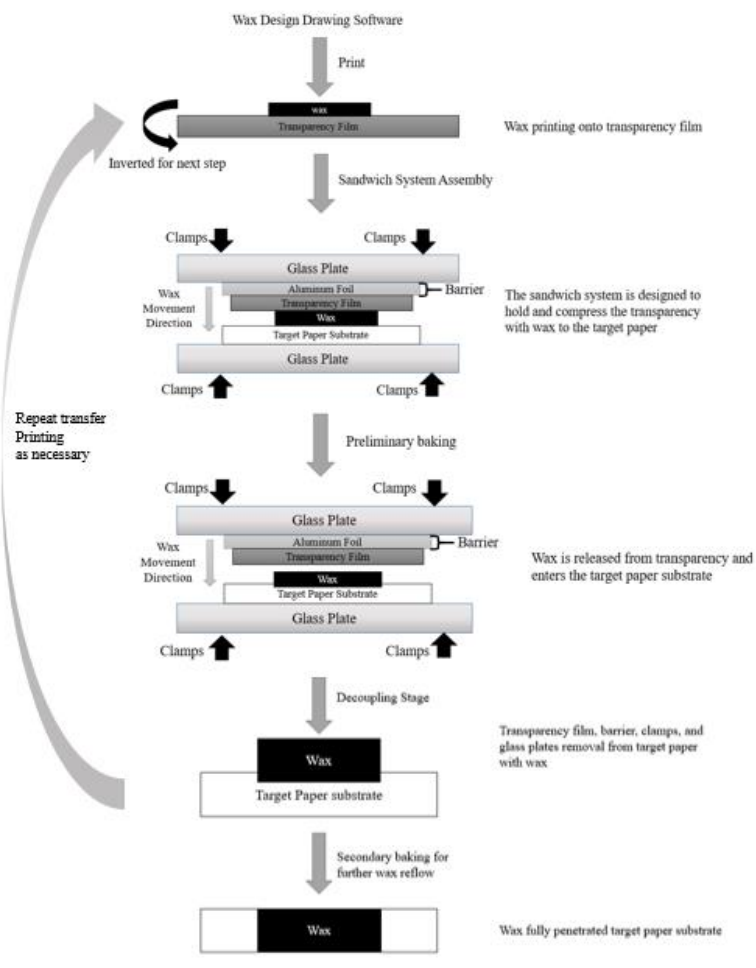


Figure 1: A schematic illustrating the wax transfer print method. Wax transfer print can be repeated several times to add more wax to a microfluidic device.

preliminary bake, the transparency film, aluminum foil, glass plates, and clamps were decoupled and separated from the wax-printed paper substrate. The paper substrate was then baked for a second time at 150°C for 5 minutes in order to melt the wax further so that it could spread through the thickness of the paper. Figure 2 shows the three major steps. The wax transfer process can be replicated multiple times to transfer more wax to the paper substrate. In this study, the wax transfer process was repeated four times for the thicker Millipore cellulose (two transfer prints per side of the paper substrate) whereas Whatman no.1 filter paper only required one transfer print overall.

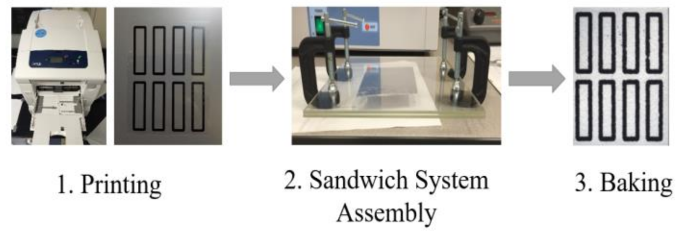


Figure 2: An illustration of the three major steps in the wax transfer process.

Wax Transfer Printing Analysis

The transfer process was evaluated using three methods: a leakage test, reservoir volume reproducibility analysis, and wax spreading analysis. The leakage test was performed to determine whether the patterned wax reservoir could retain fluid within the wax boundaries. The leakage test consisted of overfilling the wax reservoir volume by 20% with water mixed with red food dye in order to challenge the hydrophobic borders. A functional hydrophobic barrier was defined to be one that prevented water from wicking across it for 15 minutes. Images scans (300 dpi or 1600 dpi) were acquired at different times during the leakage test.

After determining wax transfer parameters that ensure functional hydrophobic barriers, we proceeded to analyze the reproducibility of the wax reservoir volume and the wax line width. Using the measurement program ImageJ, the enclosed area of each reservoir and the line width (in X and Y) was measured. ImageJ has a measurement function that allowed us to select the reservoir region and calculate the reservoir lateral area. The wax line widths were measured using ImageJ's ruler function on the reservoir top wax border (in Y) and the reservoir left wax border (in X). Figure 3 shows images of the analysis method on ImageJ.

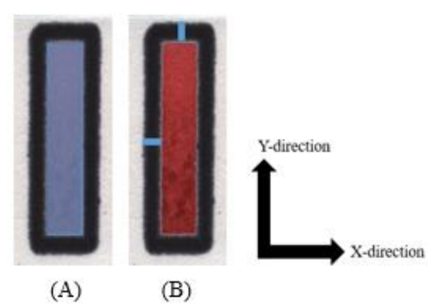
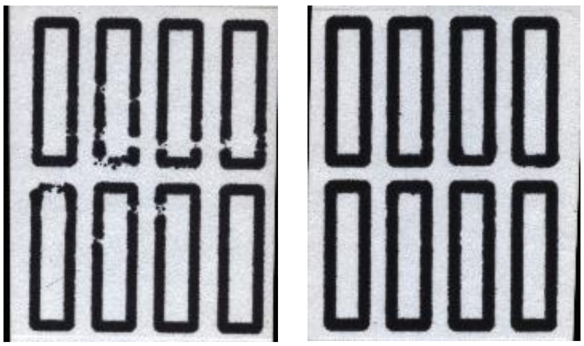


Figure 3: Analysis of reservoir volume and wax line width using ImageJ. (A) The volume was estimated by finding the area of the rectangle and multiplying by the thickness. (B) The line width was measured using the reservoir's left and top boundary.

RESULTS & DICUSSIONS

Identifying Key Transfer Parameters

The nominal wax line width and the number of transfer prints on a paper substrate were the key parameters that affected the amount of wax entering the paper. Varying the nominal line width can incrementally add wax to the paper substrate. Replicating a transfer print adds an additional layer of wax to the paper substrate. Figure 4 shows how multiple transfer prints can improve reservoir formation. Due to variation between papers, creating a wax transfer protocol for all materials is not feasible. For example, more wax is required to fill Millipore cellulose pores than Whatman no.1 filter paper because of their difference in capacity. The key transfer parameters must be tuned to the paper in order to create hydrophobic barriers. After identifying these key transfer parameters for Whatman no.1 filter paper, this study shifted its focused to study wax printing on Millipore cellulose.



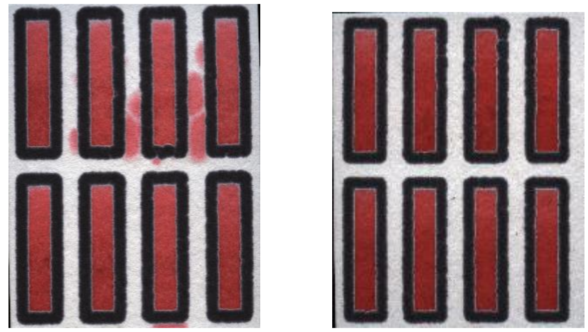
(A) (B)

Figure 4: A visualization of how multiple transfer prints help form functional hydrophobic barriers. (A) One transfer print results in incomplete reservoir formations. (B) Two transfer prints, which doubled the amount of wax entering the paper substrate, resulted in complete barrier formation.

Transfer Wax Printing Analysis

Leakage Test Analysis

The parameters necessary to create functional hydrophobic barriers using Millipore cellulose had a nominal wax line width of 1000 μm with two transfer prints per side (four transfer prints overall). We conducted six bake trials at these conditions; overall, N=48 reservoirs were tested for leakage. At these conditions, the average reservoir volume was 140 μL. During the leakage test, 169 μL of water mixed with red food dye was administer to each reservoir. After fifteen minutes, all 48 reservoirs revealed no sign of leakage despite being overfilled, giving a 100% success rate. Varied line widths were tested as well. At 600 μm with two transfer prints per side condition, two out of eight reservoirs leaked during the test (75% success rate). At 800 μm with two transfer prints per side condition, 40 reservoirs were tested and three reservoirs leaked overall (92.5% success rate). The number of transfer prints was varied as well (Supporting Information). It should be noted that a minimum of one transfer print per side (two transfer prints overall) was necessary to create at least one functional hydrophobic reservoir for Millipore cellulose. Using only one transfer print overall resulted in a 0% success rate, despite increasing the line width.



(A) (B)

Figure 5: Leakage test examples are shown above. (A) Reservoirs with unsuccessful hydrophobic barrier formation resulted in water with red dye outside the barriers. (B) Reservoirs with successful hydrophobic barrier formation resulted in the retention of water within the barrier.

Reservoir Volume Reproducibility

Reproducibility was defined to be the variation arising when an experiment was repeated under the same conditions[5]. To evaluate the reservoir volume reproducibility after confirming transfer parameters for Millipore cellulose (1000 μm with two transfer prints per side), each trial was scanned at 1600 dpi and analyzed more closely using ImageJ. The 1st trial had the highest average reservoir volume: 135.5 μL with a 1.03% coefficient of variation (CV) reservoir volume. The 4th trial had the lowest average reservoir volume:123 μL with a 1.26% CV. Across all trials, the average volume was 128 μL with a 4.04% CV. These numbers suggest that within a trial, each reservoir's volume varied between 1 to 2 μL. With low CV values, this is a good indication that any mistakes made during the fabrication process was accounted for across all reservoirs in a trial. However, when compared across all trials, each reservoir volume varied by ~5 μL. This was probably due to an alignment issue between each trial when overlaying the second transparency film over the first transfer print reservoirs. If not aligned exactly, the wax would spread into the reservoir, giving the reservoir a smaller working volume. Figure 6 shows all the trials and the average next to each other. It should be noted that the CV amongst all trials was still low. For most microfluidic devices, especially for microfluidic devices used in low-resource setting, this would be an acceptable range of variation.

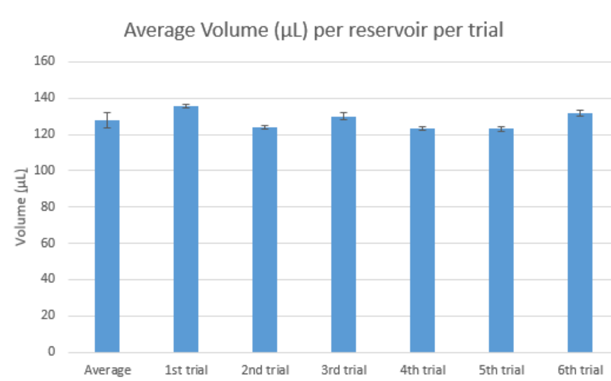


Figure 6: A graph of the average reservoir volume per trial. Error bars on the graph show 95% confidence interval. The variation in the average volume between trials was larger than in a trial.

Wax Spread Analysis

The wax spread analysis allows for the possibility to design reservoirs that account for the wax spreading in order to fabricate a more accurate volume. The nominal wax line width was 1000 μm and it spread to ~3500 µm after the baking process, which affected the reservoir volume. ImageJ analysis revealed that the average X-directional wax spreading among all trials had an average spread of ~3500 µm with a ~180 µm 95% CI while the Y-directional wax spreading among all trials had an average spread of ~3600µm with a ~160 µm CI. A two-tailed paired t-test analysis showed a significant difference (p=0.005) between the X-directional versus Y-directional wax spreading indicating that Millipore cellulose orientation affects how wax spreads[1]. It should also be noted that reservoirs with a higher degree of wax spreading correspond to smaller working volumes, while a smaller degree of wax spreading corresponds to reservoirs with larger volumes. This indicates that alignment was important for the fabrication of reservoirs with the same working volume across several bake trials. Figure 7 shows the line width spread in both the X- and Y-direction for all trials, as well as the average wax line width spread between trials.

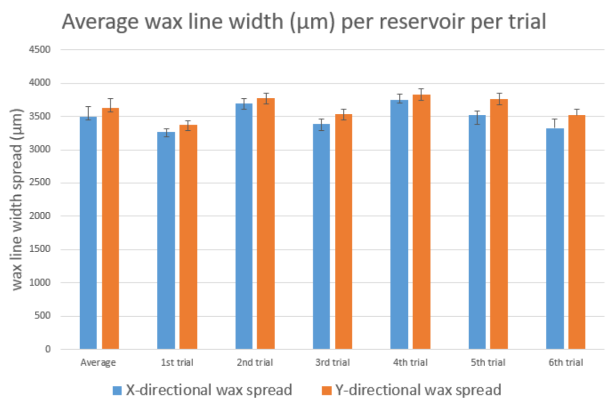


Figure 7: A graph showing the difference between X- and Y-directional wax spread amongst all trials. Each bake trial had relatively low error bars. However, the largest error bar came when all the trials was averaged indicating a fabrication inconsistency.

Transfer Printing Applications

In this study, we demonstrated wax transfer printing onto Millipore cellulose. Other papers that are too thick, too small or large, or too rough for the printer to handle can use the proposed transfer method to create wax patterns on the material. However, the proposed transfer printing method has several favorable applications outside of processing materials: (1) the wax-printed transparency film is easy to store and transport, so the transfer process can be conducted in places where a wax printer is not available[4]; (2) it allows for after-process modification if the user wants to add more wax placed onto a paper; (3) it potentially allows wax printing to occur on porous substrates outside of paper[4]. Overall, this method is appealing to the paper microfluidics field because it is a simple, rapid, and inexpensive method for fabricating devices. This method does not require a clean room, special equipment and power resources, or the usage of organic solvents[5]. This transfer method will be especially suited to microfluidic devices with low-resolution requirements[4].

Next Step

One next step of this study would be to develop a device that improves alignment between transfer prints and reduces variation in wax patterns. An alignment device would ensure that a minimal amount of human-error would affect the fabrication process. Another next step would be to implement this process in an application for low resource settings. Also, new materials could be used to further test the wax transfer print capabilities. Materials like glass fiber and cotton are widely used in the microfluidic field because their properties differ from Whatman no.1 filter paper. Identifying the minimum transfer parameters for these materials would be useful for researchers that would like to pattern-wax on these materials to create devices in their research

4. CONCLUSION

This study demonstrates a relatively simple, rapid, and inexpensive method to conduct wax transfer printing on materials that cannot be processed by a wax printer. The method requires a transparency film that acts as an intermediate template to transfer from the printer onto a desired paper substrate. The method is demonstrated to produce reproducible wax patterns on a thick cellulose substrate.

5.0 ASSOCIATED CONTACT

Supplementary Information

---See attached document---

6.0 ACKNOWLEDGEMENTS

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7.0 REFERENCES

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Supporting Information

Transfer Wax Printing Characterization: Expanding Wax Printing Capability to Various Paper-types

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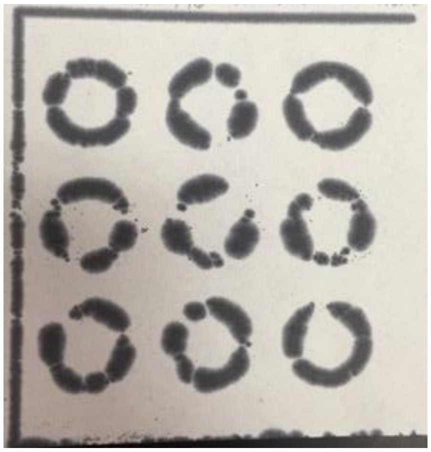
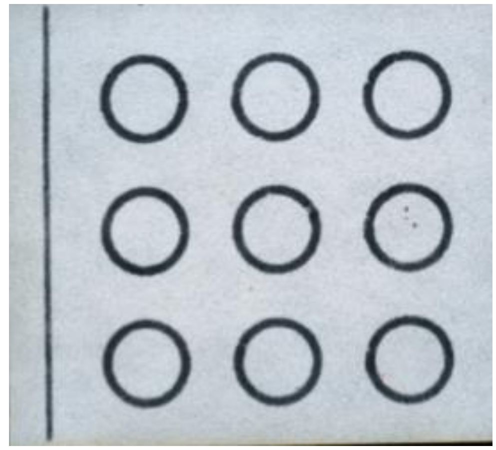
Table of Contents:

I. Eliminating Transparency Residue Issue



(A)



(B)

Figure S1: (A) depicts an image without the aluminum foil barrier. (B) depicts an image with the usage of foil as a barrier. It was speculated that the transparency residue caused non-conformal contact between the glass plates and the coated transparency film side. This in turn affected the wax transfer process because it didn’t allow wax transfer condition where the wax could not make good contact with the target paper.

II. Transfer Print-Leakage Test

2x Transfer Prints (1x transfer prints per side)			
Line width	**Number of reservoir**	**Leak-proof reservoir**	**Success Rate**
1400um	16	3	81%
1580um	8	6	75%
2000um	40	32	80%

4x Transfer Prints (2x transfer prints per side)			
Line width	**Number of reservoirs**	**Leak-proof reservoirs**	**Success Rate**
600um	8	6	75%
800um	40	37	92.5%
1000um	48	48	100%
1400um	8	8	100%
2000um	40	40	100%

Figure S2: Data tables that represents all the variables tested during the leakage test.

III. Channel Volume Data

1000um trials	volume, avg (uL)	STD (uL)	C.O.V (%)	Overcapacity (%)
Average	127.89	5.16	4.04	32.1
1st trial	135.42	1.39	1.03	24.7
2nd trial	123.93	1.43	1.15	36.4
3rd trial	130.00	2.38	1.83	30.0
4th trial	123.31	0.89	0.72	37.1
5th trial	123.17	1.56	1.26	37.2
6th trial	131.51	2.12	1.61	28.5

Figure S3: The data used to create the graph shown in Figure 6.

IV. Channel Wax Spread Data

Average Wax Line width per channel per bake						
	X-directional wax spread			Y-directional wax spread		
trials	length (um)	Standard deviation (um)	Confidence interval (95%)	length (um)	Standard deviation (um)	Confidence Interval (95%)
Average	3490.6	198.9	159.2	3629.731	179.3	143.5
1st	3271.3	57.5	48.1	3372.9	69.5	58.1
2nd	3695.7	85.9	71.8	3768.7	102.4	85.6
3rd	3381.4	97.4	81.4	3537.0	92.8	77.6
4th	3749.7	106.9	89.3	3825.9	103.3	86.4
5th	3521.1	65.0	54.4	3756.0	103.8	86.8
6th	3324.2	164.9	137.8	3517.9	103.8	86.8

Figure S4: Data used to create the graph in Figure 7

t-Test: Paired Two Sample for Means		
Results	X-directional	Y-directional
Mean	3490.56	3629.73
Variance	39562.83	32162.87
Observations	6	6
Pearson Correlation	0.943	
Hypothesized Mean Difference	0	
df	5	
t Stat	-5.120	
P(T<=t) one-tail	0.002	
t Critical one-tail	2.015	
P(T<=t) two-tail	0.004	
t Critical two-tail	2.571	

Figure S5: The paired t-test analysis data used to show the significant difference between X- and Y-directional wax spread.