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## Using printer ink color to control the behavior of paper microfluidics

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Paper microfluidic devices (including lateral flow assays) offer an excellent combination of utility and low cost. Many paper microfluidic devices are fabricated using the Xerox ColorQube line of commercial wax-based color printers; the wax ink serves as a hydrophobic barrier to fluid flow. These printers are capable of depositing four different colors of ink, cyan (C), magenta (M), yellow (Y), and black (K), plus 11 combinations of these colors (CM, CY, CK, MY, MK, YK, CMY, CMK, CYK, MYK, and CMYK), although most researchers use only black ink to print paper microfluidic devices. Recently, as part of a project to develop a computer-aided design framework for use with paper microfluidics devices, we unexpectedly observed that different colors of wax ink behave differently in paper microfluidics. We found that among the single colors of ink, black ink actually had the most barrier failures, and magenta ink had the fewest barrier failures. In addition, some combinations of colors performed even better than magenta: the combinations CY, MK, YK, CMY, CYK and MYK had no barrier failures in our study. We also found that the printer delivers significantly different amounts of ink to the paper for the different color combinations, and in general, the color combinations that formed the strongest barriers to fluid flow were the ones that had the most ink delivered to the paper. This suggests that by simply weighing paper samples printed with all 15 combinations of colors, one can easily find the color combinations most likely to form a strong barrier for a given printer. Finally, to show that deliberate choices of ink colors can actually be used to create new functions in paper microfluidics, we designed and tested a new color-based “antifuse” structure that protects paper microfluidic devices from a typical operator error (addition of too much fluid to the device). Our results provide a set of color choice guidelines that designers can use to control the behavior of their paper microfluidics.

### Introduction

Millions of people die every year from communicable diseases like AIDS, tuberculosis, respiratory infections, enteric infections, and malaria. Nearly all of these deaths occur in developing countries.<sup>1</sup> Most of these diseases can be successfully managed or cured if they are diagnosed in time, but developing diagnostics for use in resource-limited settings is challenging. In order for a point-of-care diagnostic to be successfully used in resource-limited settings, the World Health Organization recognized that the diagnostic should be:

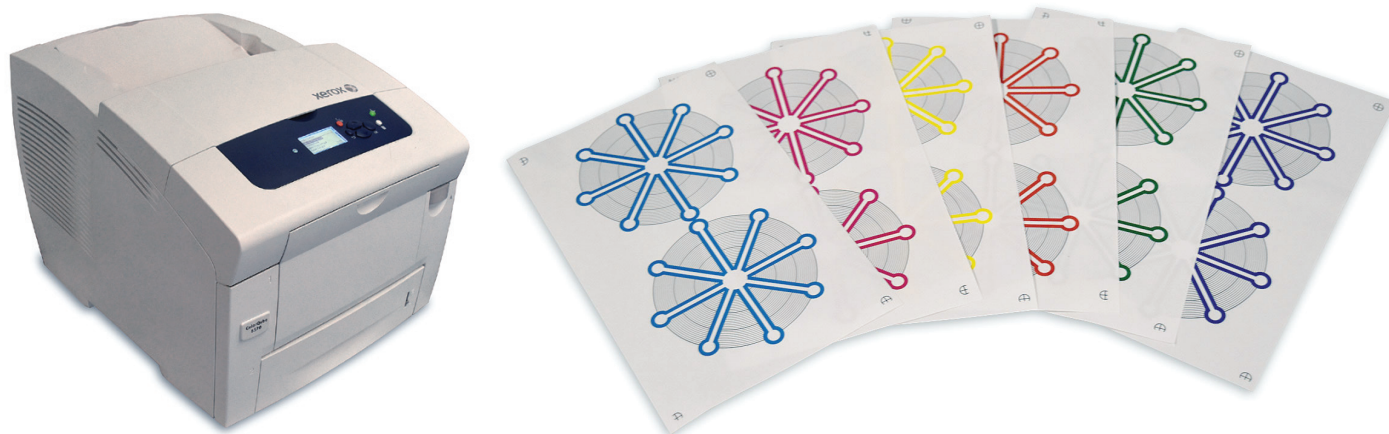
- A: Affordable by those at risk of infection
- S: Sensitive (few false-negatives)

- S: Specific (few false-positives)
- U: User-friendly (requiring minimal training)
- R: Rapid (treatment at first visit) and robust (no refrigerated storage)
- E: Equipment-free (no additional equipment needed for use)
- D: Delivered to those who need it (small and portable)<sup>1</sup>

No single diagnostic technology satisfies all of these “AS-SURED” criteria, but paper microfluidics (including lateral flow assays) may come the closest.<sup>2</sup> In paper microfluidics, a sample flows through a series of processing and analysis steps inside porous paper channels. This flow is driven by capillary forces (wicking) and requires no external valves or pumps to control it; this significantly reduces the cost and size of paper microfluidic devices compared to traditional laboratory diagnostic instruments. Perhaps the most common paper microfluidic device is the simple lateral-flow home pregnancy test, although much more complicated paper microfluidics have been developed that in-

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**Fig. 1** (Left) Xerox ColorQube wax-based color printers (like this ColorQube 8570 model) are routinely used for creating paper microfluidic devices. (Right) Paper microfluidic test devices for six of the 15 different wax ink color combinations we tested (from left): cyan, magenta, yellow, magenta + yellow, cyan + yellow, and cyan + magenta.

clude complex networks of paper channels,<sup>3,4</sup> paper-based valves and other flow-control structures,<sup>5,6</sup> and even origami-inspired designs.<sup>7,8</sup>

One of the most common techniques for fabricating paper microfluidic devices utilizes the Xerox ColorQube line of color office printers. Instead of liquid ink or powdered toner, ColorQube printers (like the one shown in Figure 1) contain solid blocks of wax-based ink, which the printer melts and deposits on the paper. Chandler *et al.*<sup>9</sup> showed that since this wax ink is hydrophobic, it can be used to make channel walls and define fluid paths in paper microfluidics.

Recently, as part of a project to develop a computer-aided design (CAD) framework for use with paper microfluidics,<sup>10</sup> we unexpectedly observed that the different colors of ColorQube wax ink behave differently in paper microfluidics. This observation may have gone unnoticed by previous researchers because even though ColorQube printers can print wax of four different colors (cyan, magenta, yellow, and black) plus all combinations of these colors, most researchers use only the black ink when printing their paper microfluidic devices. In one notable exception, Taudte *et al.*<sup>11</sup> used a few different ink colors in their paper microfluidic devices for detecting explosive materials, and concluded that magenta ink served as a more robust barrier to fluid flow than black ink. However, to the best of our knowledge, no one has systematically explored all 15 possible combinations of cyan, magenta, yellow, and black that can be delivered by ColorQube printers, or identified color combinations with behaviors that make them suitable for functions other than fluid barriers, or offered a possible explanation for the different behaviors of different ink colors.

In this work, we systematically explore the effect of ColorQube wax ink color on paper microfluidic device behavior. This study required the creation of a large number of different paper microfluidic device designs, and we leveraged our paper microfluidics CAD framework to automate and expedite the generation of these device designs.<sup>10</sup> Our findings provide a set of rules on color selection that researchers can use to easily control the behavior of their paper microfluidics. We also provide a explanation

for why different colors behave differently, and demonstrate that a series of simple mass measurements can be used to determine which color combinations will likely provide the strongest barriers to fluid flow for a given printer. Finally, we demonstrate a useful new paper microfluidic component—a paper microfluidic “antifuse”—that uses different ink colors to create the desired behavior.

## Materials and Methods

### Overview of color printing with the CMYK model

Xerox ColorQube printers use solid blocks of wax-based ink in four different colors: cyan (C), magenta (M), yellow (Y), and black (K). This means that the printers use the *CMYK model*, a subtractive color model that uses secondary colors to reproduce the full range of colors.<sup>12</sup> To reproduce primary colors, CMYK printers generally combine two of the secondary colors (*e.g.*, magenta and yellow are combined to form red; see Figure 1 for examples). The black ink is used to produce darker shades of the colors in the CMYK model but is not generally used by itself where a full black color is desired when printing. Rather, a percentage of cyan is also combined with black to create a more opaque black color. Finally, a combination of all four inks known as “registration” black is used for aligning inks when using presses that print each color separately. In summary, 15 different combinations of ink colors can be generated by these printers: C, M, Y, K, CM, CY, CK, MY, MK, YK, CMY, CMK, CYK, MYK, and CMYK. By controlling the color of a device feature in a graphical design program, the user can easily create paper microfluidic device features in any of these color combinations.

### Automated design of paper microfluidic devices

Instead of designing our paper microfluidic devices by hand using graphic design software, in this work we used our computer-aided design (CAD) framework<sup>10</sup> to automatically generate the necessary device designs. This framework seeks to accelerate the development of new paper microfluidic devices and reduce the

material required to make these devices. Developers can use the framework to prototype, dynamically generate, and test new designs. These designs may account for the effects of different environmental conditions, physical substrates, and fluid conditions, providing the designer with a greater understanding of how these physical factors influence the behavior of a microfluidic assay. The framework includes a library of paper microfluidic components which can be rapidly assembled into a desired microfluidic device design. Once the components are assembled, the software framework renders the microfluidic device using established file formats such as PDF, DXF, and SVG, which may then be used by printers, plotters, paper cutters, and other output devices.

The framework contains a library of components for developing devices, as well as pre-defined devices that are parameterized to allow for quick alteration of specifications. In this work, we used the library's "bullseye" calibration device shown in Figure 1. This design provides the user with a radial array of channels that connect a central "source" to individual "sinks" around the edges of the device as well as markers alongside the channels to facilitate measurements of fluid travel. The user specifies the various parameters of the desired "bullseye" device, including the number, width, and angle of the channels, the diameters of the sources and sinks, and the widths and colors of the wax ink channel barriers. Our software framework then generates a graphics file ready to print on the color printer. In this manner, we easily generated all 48 different "bullseye" device designs used in this study in less than a second per device. Over the course of this study, we also added the "antifuse" design (described below) as a new component in the library to facilitate the inclusion of this component in future paper microfluidics.

### Fabricating paper microfluidic devices

Currently, Xerox ColorQube printers are the only wax-ink-based printers on the market. Xerox has sold 12 different models of ColorQube printers, and the exact compositions of the inks used by the printers are trade secrets. However, close inspection of the inks' Material Safety Data Sheets (MSDSs)<sup>13–15</sup> reveals that the inks used by all 12 of the ColorQube printers share the same basic chemical composition (by weight):

- 50–60% paraffin wax
- 10–20% resin
- 0–10% blue dye
- 0–10% red dye
- 0–10% yellow dye
- 0–10% black dye

In other words, the inks used by all 12 models of ColorQube printers are mostly paraffin wax and resin, with the remainder consisting of one or more dyes that are specific to each ink color. Additionally, the other ink data provided by the MSDSs (hazards, melting point, flash point, solubility, specific gravity, chemical regulatory data, etc.) are all identical across the different ColorCube

models. This suggests that the composition of each ink color is very similar (and possibly identical) across the 12 different ColorQube printers, although the exact compositions are trade secrets.

In this work, we used a Xerox ColorQube 8570 printer (Figure 1) connected via USB to a PC loaded with our CAD framework running on the Linux operating system. We printed the generated device designs on "Lab Nerd 101" fast qualitative filter paper (Amazon.com). Since this filter paper comes from the manufacturer as 24 cm diameter circles but the ColorQube printer requires rectangular paper for printing, we cut each sheet down to a 20 cm × 12 cm rectangle for manually feeding into the printer. This provided enough space for two separate paper microfluidic test devices per sheet. After printing, each pair of devices was heated over a laboratory hotplate to re-melt the wax ink and form a hydrophobic barrier across the full thickness of the paper. The hotplate was pre-heated to 166 °C (as measured using an infrared thermometer), then each printed paper sample was held 5–7 cm above the heating element (with the printed side facing upward) until melting was observed in the wax ink (ink color faded and the edges of the ink features blurred). At this point, the Bullseye Calibration Devices were ready to use in experiments. The "antifuse" test paper microfluidic devices had self-adhesive PCR plate sealing film (Microseal B; Bio-Rad, Hercules, CA) applied to the backside (non-ink side) of the paper before use; this tape keeps fluid from flowing through the backside of the paper.

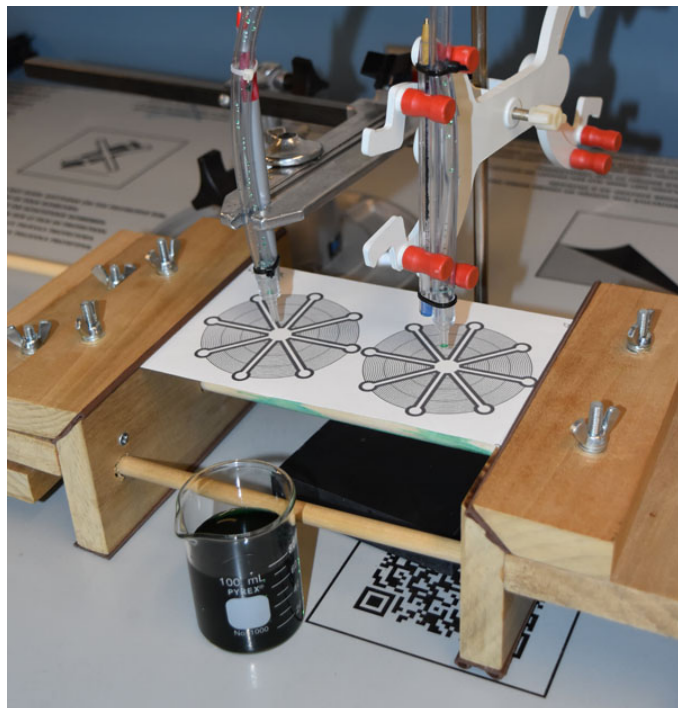
### Testing paper microfluidic devices

The "bullseye" paper microfluidic devices (shown in Figure 1) were tested using a custom rig built out of finished lumber (Figure 2). Each paper device was clamped into the leveled rig and suspended from the edges to eliminate any contact between the work surface and the backside of the paper device. The devices were tested using deionized water containing a small amount of food coloring (3 drops per 50 mL) delivered to the paper using either a pipette (for the black ink color studies) or a mounted low-flow-rate nozzle (Antelco; Longwood, FL; for the color ink studies, shown in Figure 2; the nozzles delivered 300 μL of fluid to each paper microfluidic device at a rate of 200 μL per second). Along with photographing each device during fluid delivery, we also recorded the time when fluid reached the first sink and the time when fluid reached the eighth and final sink.

The "antifuse" paper microfluidic devices were tested on a bench top. A pipette was used to repeatedly deliver 50 μL volumes of fluid to the source reservoir of the device until flow across a wax ink barrier was observed.

### Analysis of the amount of wax ink delivered by printer

For studies of the amount of each ColorQube ink deposited on the paper by the printer, a laboratory analytical balance was used to weigh paper samples. Ink mass was determined by first measuring the pre-print mass of a paper substrate along with a binder clip used to hold the paper in a cylindrical shape to accommodate the interior dimensions of the balance enclosure. The mass of the clip was then subtracted from subsequent measurements.



**Fig. 2** Custom-built test rig for dispensing known volumes of fluid at known flow rates to paper microfluidic devices.

A 17 cm by 10 cm solid rectangle of ink was then printed on the paper. After printing, the paper was weighed again and the two mass measurements were subtracted to determine the mass of the ink deposited by the printer. Dividing this mass by 170 cm<sup>2</sup> (the area of the ink rectangle) yields the mass of ink deposited per unit area. This measurement was repeated for all 15 solid colors (single colors and combinations) that can be generated by the printer.

## Results and discussion

### Barrier width testing with black ink

Preliminary experiments were performed to determine the necessary ink barrier width for reliable fluid containment using black ColorQube ink (which is the usual ink color used in paper microfluidics). We used the “bullseye” device from our framework library, configured with 8 radial channels, 2 mm wide channels, 6 mm diameter sinks, 12 mm diameter sources, and 86 mm overall diameter. Ink barrier widths were varied from 1.0 to 2.0 mm (Fig. 3), and 16 devices were tested for each width (a total of 48 different tests). A pipette was used to deliver 300  $\mu$ L of dyed water to the central source of each “bullseye” pattern, and the fluid was allowed to travel outward to the eight sinks on the perimeter, stopping when the fluid had reached one or more of the sinks. Failures were categorized as either “flow through barrier” (when fluid wicked through the ink-impregnated barrier) or “flow over barrier” (when fluid flowed across the top of the ink barrier).

Results from our black barrier width study are summarized in Table 1 row K, and typical results are shown in Figure 3. We found that the thinnest black ink barriers (1.0 mm thick) consistently failed by having the fluid flow over the thin barrier. At moderate

barrier thicknesses (1.5 mm), failures occurred most often due to flow through the barrier. Finally, devices with the largest barrier thicknesses (2.0 mm) exhibited the fewest total failures. Fluid took from 144 s to 488 s to reach the first of the eight sinks, with a median time of 392 s. Fluid took from 157 s to 560 s to reach the last sink, with a median time of 443 s.

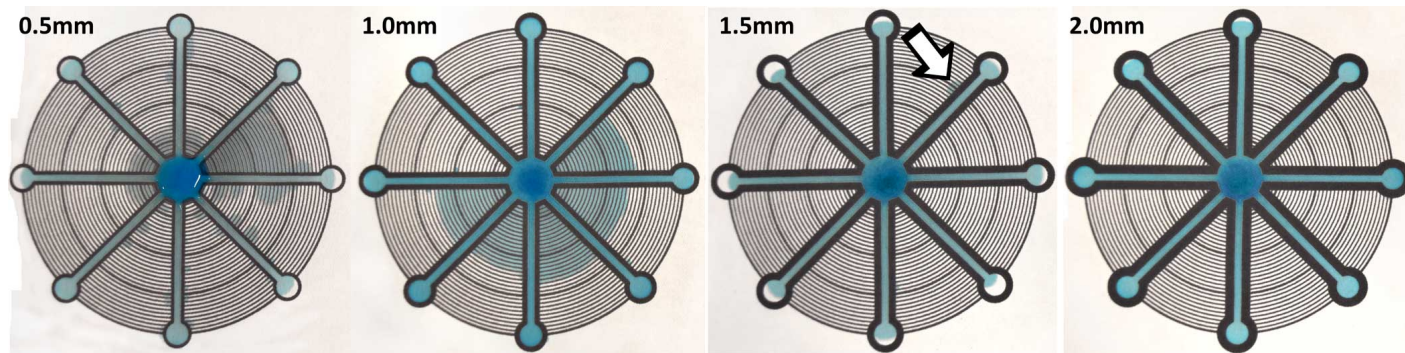
### Barrier color testing with individual cyan, magenta, and yellow inks

We then repeated the barrier tests shown in Figure 3, but this time using the other individual ink colors provided by ColorQube printers (cyan, magenta, and yellow) instead of black. The results summarized in Table 1 (lines C, M, and Y) reveal significant differences in how the different individual ink colors perform as barriers to fluid flow. Interestingly, black ink (K), which is used most often in wax-printed paper microfluidic devices, actually performed the worst of all the single-color barriers in every barrier width, with 21 failures out of 48 total tests or a 44% failure rate. Magenta ink (M) performed the best, with only three failures observed in the thinnest barriers and no failures in the other barrier thicknesses (a combined failure rate of only 6%). These results are consistent with the observations of Taudte *et al.*<sup>11</sup> and suggest that **for maximum resistance to unwanted fluid flow through or across the ColorQube wax ink barriers, when choosing one of the printer’s four individual ink colors, creators of microfluidic devices should use magenta ink instead of black.**

We also observed an unusual behavior in test devices with yellow barriers. Table 1 shows that while no “flow over barrier” failures were observed in the thicker black, cyan, and magenta barriers, several “flow over barrier” failures were observed in the thicker yellow barriers. Figure 4 shows typical yellow “bullseye” devices both before and after a “flow over barrier” failure; the blue fluid is flowing across two millimeters of yellow wax ink barrier. These observations suggest that the surface of paper impregnated with yellow wax ink may be less hydrophobic than the other colors. These observations also suggest that the distinctive failure mode of yellow ink barriers could be used by designers to impart certain behaviors to paper microfluidic devices, an idea we test later in this work.

### Barrier color testing with combinations of ink colors

As noted above, ColorQube printers are capable of printing colors other than cyan, magenta, yellow, and black by simultaneously delivering *combinations* of two or more colors to the paper. We hypothesized that these combinations may result in more wax ink being delivered to the paper (therefore creating a more effective barrier to fluid flow) compared to the single ink colors. To test this hypothesis, we repeated the above tests using devices made with all possible combinations of cyan, magenta, yellow, and black (CM, CY, CK, MY, MK, YK, CMY, CMK, CYK, MYK, and CMYK). In each combination, each color is specified at 100% density, meaning that the printer is instructed to deliver as much of each color as possible. This means that all color combinations that include black will appear black when printed, but will still



**Fig. 3** Sample results from experiments to determine reliable barrier thickness for black ColorQube ink using our software framework's "bullseye" test device. Consistent barrier failure occurred at black barrier widths of 1.0 mm and smaller. The 1.5 mm barrier did a much better job of containing fluid, but some failures were still observed (as indicated by arrow). Ultimately, a 2.0 mm barrier width was found to have the fewest barrier failures.

contain the additional ink colors.

The results in Table 1 show that most devices containing multiple color barriers fared better than the single-color barriers. Of the 11 possible color combinations, 6 of them (CY, MK, YK, CMY, CYK, and MYK) had no flow failures at any of the barrier widths tested. **These color combinations are excellent choices for robust barriers, especially at thinner barrier widths.** They outperformed all of the single-color barriers. An additional three combinations (CK, CM, and MY) failed only for the thinnest barrier, a performance on par with magenta (the best single-color barrier).

However, two combinations of wax colors performed far worse than the other combinations. Specifically, all but one of the 48 test devices printed using CMK barriers suffered from "flow through barrier" failures (a staggering 98% failure rate) and 14 of the 48 test devices printed using CMYK barriers suffered the same failure (a 29% failure rate). We hypothesized that these failures might be attributed to variation in the amount of ink the printer deposits in the paper when printing these color combinations, a hypothesis we test in the next section.

### Measuring the mass of ink deposited on the paper

To gain insights into why different ink color combinations behave dramatically differently in paper microfluidic devices, we measured the mass of ink being deposited by the printer for each single color and color combination, both before and after heating. The results (Table 2 and Figure 5A) reveal significant variation in the total amount of ink delivered by the printer for different color combinations. The color combination with the largest mass of ink delivered to the paper (K; 1.91 mg/cm<sup>2</sup>), has over twice the mass of the combination with the smallest mass of ink delivered (CMK; 0.89 mg/cm<sup>2</sup>). Also, we observed that a significant amount of this ink mass is lost when the paper is heated. A plot of pre-heating ink mass vs. post-heating ink mass (Figure 5A) shows that each color combination lost roughly the same amount of mass during heating (about 0.3 mg/cm<sup>2</sup>). This suggests that for each color combination, the printed ink contains about 0.3 mg/cm<sup>2</sup> of volatile components that are lost during heating, plus a variable amount of less-volatile pigments and waxes that remain on the paper after heating.

Since paper microfluidic devices with wax inks must be heat-

treated before use, we focused our remaining analysis on post-heat-treatment ink masses. Figure 5B plots the rate of barrier failures vs. post-heating ink mass for all 15 ink color combinations. The results show that (with one exception) **the color combinations with largest mass of ink delivered to the paper have the lowest rates of barrier failure.** The one exception to this trend is black ink (K), which had poor barrier performance despite having the second-highest post-heating ink mass. This suggests that the black ink contains a relatively large amount of pigment (necessary for a solid black color), and this pigment reduces the color's effectiveness as a barrier to fluid flow, perhaps by reducing the hydrophobicity of the ink, or occupying space that would otherwise be filled by hydrophobic wax.

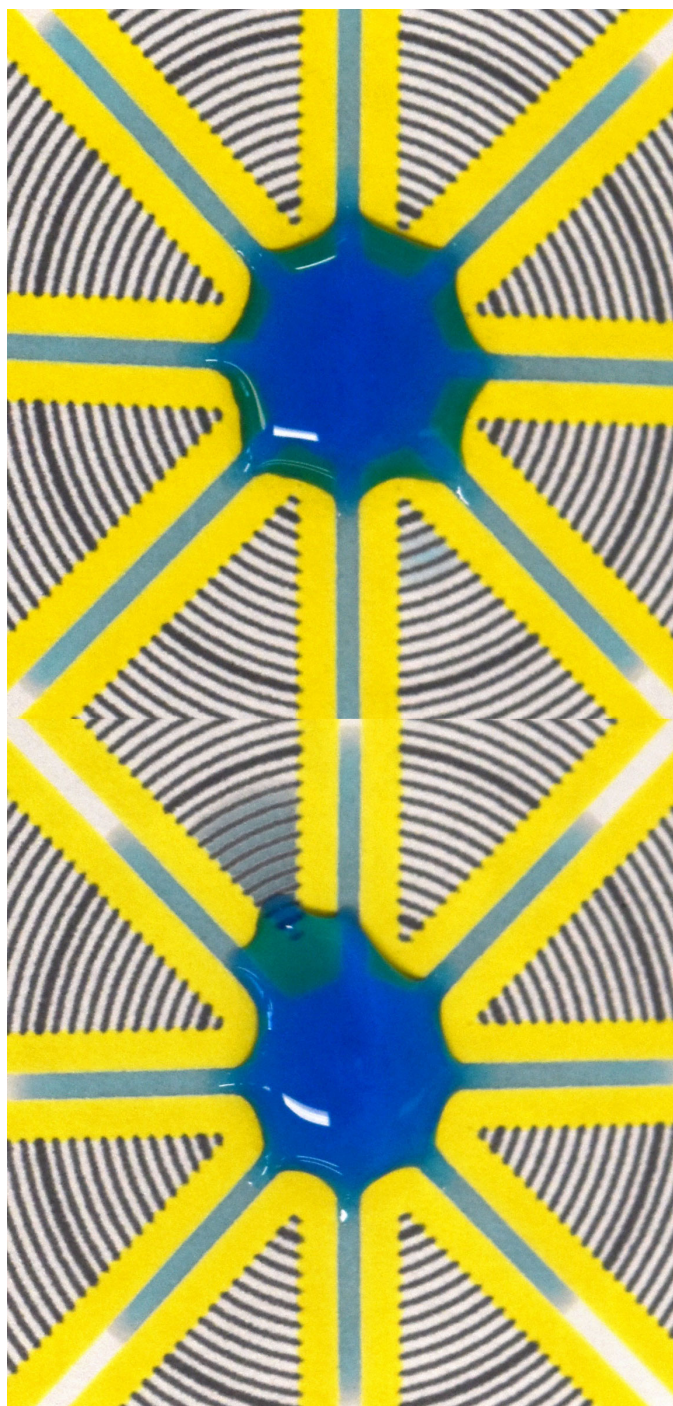
### The paper microfluidic "antifuse"

Finally, to show that deliberate choices of ColorQube ink colors can actually be used to create new functions in paper microfluidics, we used our findings to design and test a paper microfluidic "antifuse," an ink structure that deals with excess fluid in a controlled manner. In electronics, an antifuse is a component that functions as the opposite of a fuse; it normally provides a high resistance to electric current, but becomes low resistance when the voltage across it exceeds a certain level. Our paper microfluidic antifuse functions similarly by providing an initial hydrophobic barrier that resists fluid flow until a certain volume of fluid is delivered to the component, at which point the antifuse becomes a low resistance path that carries excess fluid to a desired location.

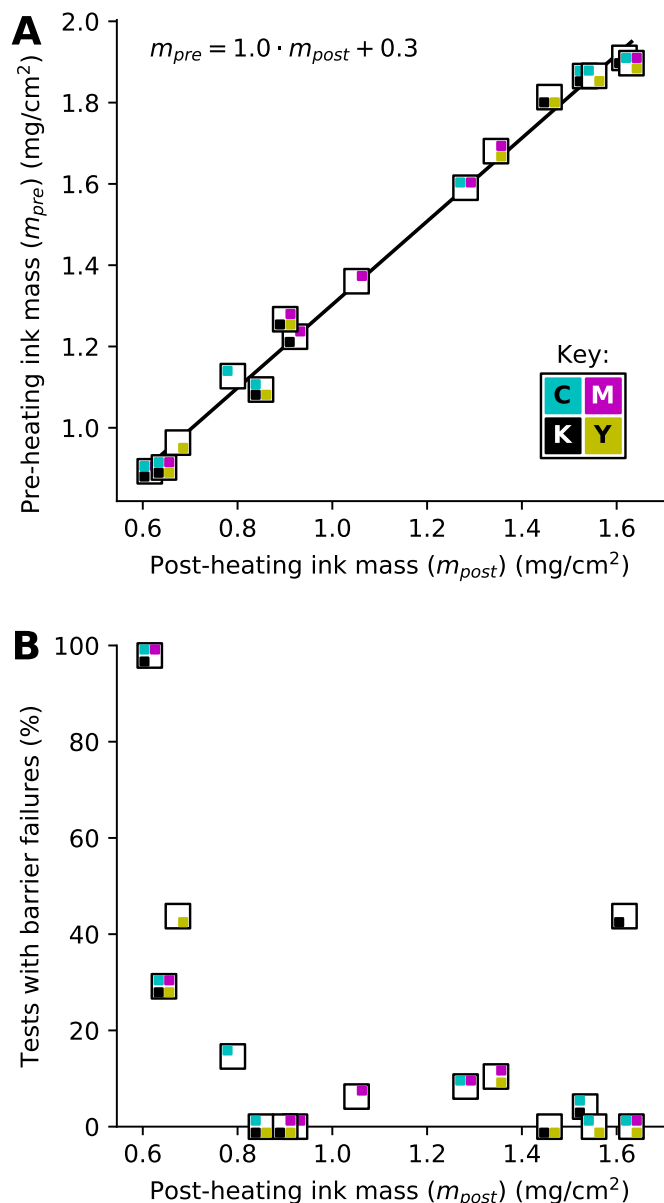
The main feature in the antifuse test device shown in Figure 6A is a horizontal channel for fluid flow; fluid is added to the circular pad on the left (marked "source") and flows by wicking through the channel and to the circular pad on the right (marked "sink"). In a real application, this horizontal channel might contain immobilized reagents that serve as readout lines in a lateral flow assay. Most of the hydrophobic barriers around this channel are magenta, chosen because magenta offers the greatest resistance to fluid flow of all the single ink colors. However, yellow ink was used for the barrier around the source pad which receives added fluid. We hypothesized that by using yellow wax ink for this antifuse barrier, we could take advantage of the tendency of yellow ink to exhibit flow-over failures like the ones shown in Figure 4.

Ink colors	Barrier width	Flow through barrier	Flow over barrier	Total failures
K	1.0 mm	0/16	6/16	6/16
	1.5 mm	11/16	0/16	11/16
	2.0 mm	4/16	0/16	4/16
C	1.0 mm	5/16	0/16	5/16
	1.5 mm	1/16	0/16	1/16
	2.0 mm	1/16	0/16	1/16
M	1.0 mm	0/16	3/16	3/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
Y	1.0 mm	6/16	5/16	11/16
	1.5 mm	3/16	2/16	5/16
	2.0 mm	0/16	5/16	5/16
CK	1.0 mm	0/16	2/16	2/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
CM	1.0 mm	2/16	2/16	4/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
CY	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
MK	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
MY	1.0 mm	0/16	5/16	5/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
YK	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
CMK	1.0 mm	16/16	0/16	16/16
	1.5 mm	16/16	0/16	16/16
	2.0 mm	15/16	0/16	15/16
CMY	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
CYK	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
MYK	1.0 mm	0/16	0/16	0/16
	1.5 mm	0/16	0/16	0/16
	2.0 mm	0/16	0/16	0/16
CMYK	1.0 mm	5/16	0/16	5/16
	1.5 mm	8/16	0/16	8/16
	2.0 mm	1/16	0/16	1/16

**Table 1** Occurrences of barrier failures using single ink colors black (K), cyan (C), magenta (M), and yellow (Y), and all combinations of these colors, at various barrier widths. “Flow through barrier” failures occurred when fluid was observed to wick through the ink-impregnated barrier and out of the channel region, and “flow over barrier” failures occurred when fluid was observed to flow across the top of the ink barrier and out of the channel region. Among single-color inks, black ink (K) performed the worst (failing in 21/48 or 44% of tests), and magenta ink (M) performed the best (failing in only 3/48 or 6% of tests). Among combinations of ink colors, the combinations CY, MK, YK, CMY, CYK, and MYK had no flow failures at any of the barrier widths tested.



**Fig. 4** Photographs of two test devices with 2.0 mm wide yellow Color-Qube ink barriers, one immediately before a “flow over barrier” failure (top) and one immediately after (bottom). Both photos were taken 2 minutes and 16 seconds into the experiment.

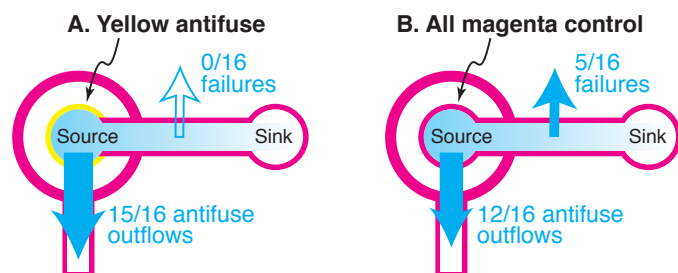


**Fig. 5** (A) Plot of the mass of ink delivered by the printer for all 15 combinations of ink colors, before (y-axis) and after (x-axis) heating the paper samples to re-melt the ink and form a hydrophobic barrier. While the total mass of ink delivered by the printer per square centimeter of paper area varies significantly with color combination, all samples lost roughly the same amount of weight during heating (about 0.3 mg/cm<sup>2</sup>). (B) Plotting the fraction of tests with barrier failures from Table 1 vs. the post-heating mass of ink delivered by the printer for the same combinations of ink. In general, color combinations with more ink delivered to the paper resulted in fewer barrier failures. The main exception to this trend was black (K), which suffers from significant barrier failures despite having one of the largest masses of ink delivered to the paper.

Colors	Pre-heating ink mass (mg cm <sup>-2</sup> )	Post-heating ink mass (mg cm <sup>-2</sup> )	Ink mass loss (mg cm <sup>-2</sup> )
K	1.91	1.62	0.29
M	1.36	1.05	0.31
C	1.13	0.79	0.34
Y	0.96	0.68	0.29
CK	1.86	1.54	0.33
CY	1.86	1.55	0.31
YK	1.81	1.46	0.35
MY	1.68	1.35	0.34
CM	1.59	1.28	0.31
MK	1.22	0.92	0.30
CMY	1.90	1.63	0.26
MYK	1.26	0.90	0.36
CYK	1.09	0.85	0.25
CMK	0.89	0.62	0.28
CMYK	0.90	0.65	0.26

**Table 2** Masses of ink deposited on the paper, for each single color (C, M, Y, and K) and combinations of colors, before and after heating the paper, as well as the mass lost due to heating. The results show a wide range in the amount of ink deposited for the different colors (ranging from 0.89 mg/cm<sup>2</sup> for CMK to 1.91 mg/cm<sup>2</sup> for K), though all colors lost roughly the same amount of mass during heating (about 0.3 mg/cm<sup>2</sup>).

Specifically, if excess fluid is added to the source pad, the fluid would flow over the yellow antifuse barrier and be contained in the large “moat” surrounded by the larger circular magenta barrier, instead of overflowing a barrier elsewhere in the device and likely invalidating the assay. In this manner, the yellow antifuse structure would enable the paper microfluidic channel to automatically recover from a typical operator error, the addition of too much fluid to the paper microfluidic device.

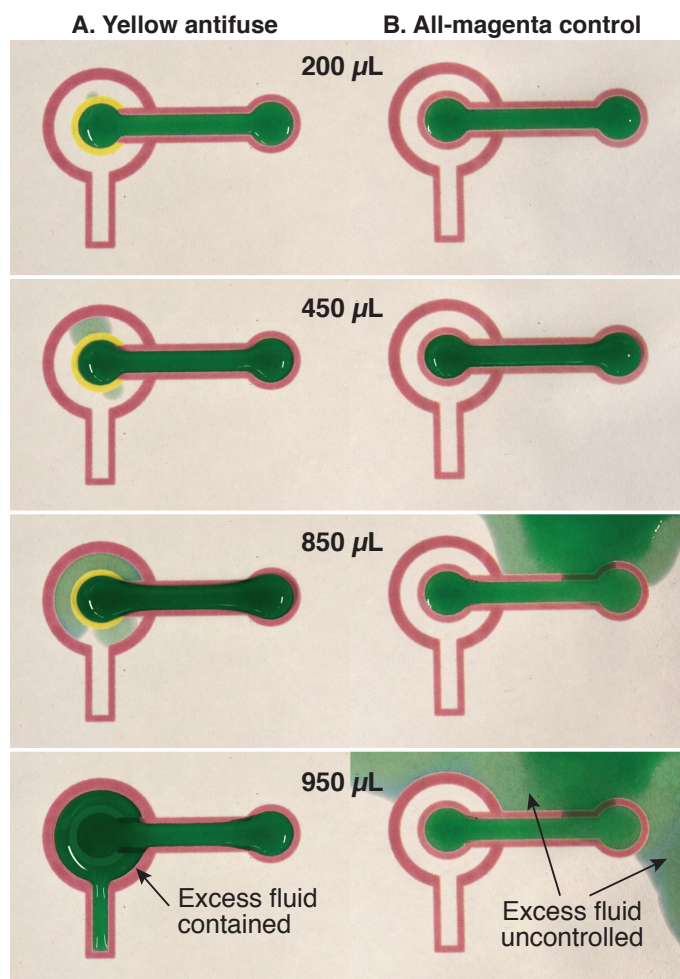


**Fig. 6** Summary of results from 32 tests of an ink-color-based “antifuse” for paper microfluidics. (A) When an operator adds excess fluid to the left-side circular pad marked “source,” the excess fluid flows over the yellow antifuse barrier and is trapped in the large circular “moat” in 15 of 16 tests, thereby protecting the rest of the device (where no failures were observed in all 16 tests). (B) In contrast, when a magenta antifuse barrier is used, excess fluid overflows into the “moat” less frequently (in 12 of 16 tests), meaning more fluid flows toward toward the “sink” pad and causes more device failures (5 of 16 tests). These results show that a yellow antifuse barrier can serve to protect paper microfluidic devices from a common operator error (the addition of excess fluid).

To test the antifuse structure, we used our software framework to create two different versions of the antifuse test device: one containing a yellow antifuse channel and expected to function as described above (Figure 6A), and one containing a magenta antifuse channel and intended to serve as an all-magenta “control” expected to fail at unpredictable locations (Figure 6B). We then fabricated 16 of each of these designs and tested each by delivering water via pipette in 20 – 50  $\mu$ L increments to the source pads while watching for either flow over the antifuse barrier or failures elsewhere in the device.

For the 16 tests with a yellow antifuse (Fig. 6A), the excess water flowed over the antifuse barrier in 15 of the 16 experiments;





**Fig. 7** Photographs of two typical antifuse test devices while adding fluid to the left-side circular source pad. As more fluid is added to the device with a yellow antifuse barrier (**A**), the excess fluid flows over the yellow antifuse and into the surrounding “moat,” protecting the rest of the device from barrier failures. In contrast, when excess fluid is added to the device with a magenta antifuse (**B**), the barrier fails catastrophically in an unpredictable location, likely invalidating the assay. These results show that yellow antifuse structures can protect a paper microfluidic device from some types of failures.

no failures occurred elsewhere in the device. This means that the yellow antifuse functioned as intended, routing excess fluid into a specified overflow area and protecting the rest of the device from barrier failures. Photographs of a typical yellow antifuse test device in operation are shown in Figure 7A; as the amount of fluid added to the Source pad increases, the flow across the yellow antifuse barrier also increases, protecting the rest of the device from barrier failures.

In contrast, for the 16 tests with a magenta antifuse, flow over the antifuse barrier was less common (occurring in 12 of the 16 experiments), meaning that more of the excess fluid flowed down the horizontal channel and caused barrier failures in 5 of the 16 experiments. The photos of a typical magenta antifuse test device in operation in Figure 7B show that when enough excess fluid is added to cause a barrier failure, the failure occurs in an unpredictable location and likely would cause a catastrophic failure of the device.

In summary, these results show that **placing yellow antifuse barriers at locations where overflow is permissible can help protect other parts of the device where overflow must be avoided.**

## Conclusions

Our results show that designers of paper microfluidic devices can use different ColorQube ink colors to impart specific behaviors to their devices. Among the single-color inks, we observed that magenta had the fewest barrier failures, a finding that is consistent with previous work.<sup>11</sup> But we also found that certain ink color combinations perform even better than magenta: the combinations CY, MK, YK, CMY, CYK and MYK had no barrier failures in our study. Therefore, for applications requiring the strongest possible hydrophobic barrier, researchers are advised to use these combinations.

This study was limited to a single model of wax-based color printer, the Xerox ColorQube 8570 printer shown in Figure 1. But based on the apparent similarity of the inks used in the different ColorQube printers, we expect that our results will apply to other ColorQube models as well. Additionally, one can predict which colors will provide the strongest fluid barriers on a new printer without repeating all of our experiments. Based on our finding that ink mass correlates with barrier effectiveness (except for K), one can easily create same-size printed samples of all 15 ink color combinations, weigh them, and determine which non-K color combinations result in the largest amount of ink delivered to the paper (and are therefore most likely to provide the strongest barrier to fluid flow).

The one notable exception to our observation that increased ColorQube ink mass results in stronger fluid barriers was black, which had one of the largest on-paper ink masses but provided one of the weakest barriers to fluid flow. Why is the behavior of black ink so different from the other colors? The exact chemical compositions of the ColorQube inks are trade secrets, and reverse-engineering Xerox’s products is beyond the scope of this work. However, if one were to predict which one of the ColorQube’s four ink colors might behave very differently than the others, that color would undoubtedly be black. Unlike cyan, magenta, and

yellow, which form a light and transparent coating on the paper to allow the reflectiveness of the underlying white paper to show through, black must form a dense and opaque layer that blocks all light from the paper. Consequently, black ink would be expected to contain a greater amount of pigments and dyes than other colors of ink, which may explain why the mass of black ink delivered to the paper is greater than the mass of almost all other colors (single and combinations). And if these black colorants are less hydrophobic than wax (or if they occupy space that would have otherwise been filled by hydrophobic wax), then it is reasonable to expect that black ColorQube ink will provide a less-hydrophobic and weaker barrier to fluid flow than the other color inks, which is exactly what we observe in this work.

Finally, our findings show that ink color selections can be used for more than just reducing barrier failures. By selecting a color with a known propensity to fail in a certain manner, we created a paper microfluidic “antifuse” that can serve as a fail-safe protection against operator error in lateral flow devices. We expect that this antifuse structure is the first of many new paper microfluidic components that utilize different ink colors to perform useful functions.

## Conflicts of interest

There are no conflicts of interest to declare.

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