We present a new method for fabricating three-dimensional paper-based fluidic devices that uses toner as a thermal adhesive to bond multiple layers of patterned paper together. The fabrication process is rapid, involves minimal equipment (a laser printer and a laminator) and produces complex channel networks with dimensions down to 1 mm. The devices can run multiple diagnostic assays on one or more samples simultaneously, can incorporate positive and negative controls and can be programmed to display the results of the assays in a variety of patterns. The patterns of the results can encode information, which could be used to identify counterfeit devices, identify samples, encrypt the results for patient privacy or monitor patient compliance.

Microfluidic paper-based analytical devices (microPADs) have attracted much attention recently for their potential applications in the area of inexpensive point-of-care diagnostics. MicroPADs are made by patterning paper into hydrophilic channels bounded by hydrophobic barriers. Three-dimensional (3D) microPADs are made by stacking layers of patterned paper in such a way that channels in adjacent layers of paper connect with each other. This technique expanded the capabilities of microPADs significantly by allowing higher channel density, and therefore function, to be incorporated into a paper-based device without significantly changing the size of the device. For example, a 3D device smaller than a credit card can distribute samples from a single sample inlet into an array of over one thousand test zones where independent assays can be performed. The main drawback of 3D microPADs was that their fabrication was relatively complicated and time consuming. The original fabrication method involved stacking layers of patterned paper and double-sided tape. Other examples of 3D devices used an external casing to hold layers of paper together or relied on manual pressure to generate contact between different layers of paper. The Phillips group recently published the first simplified method for assembling 3D microPADs using spray adhesive to permanently bond layers of paper together.

We present a new method for fabricating 3D microPADs using toner as both a thermal adhesive to bond layers of paper together and as an impermeable barrier to separate channels in adjacent layers of paper (Fig. 1). This process minimizes the number of layers of material that need to be aligned and assembled. We demonstrate that this method is rapid, technically simple to adopt, can produce features down to 1 mm in diameter (the practical size-limit of fabrication for paper-based devices) and involves only the use of a laser printer and a thermal laminator in addition to the equipment for patterning paper. The fabrication method provides several ways of programming fluid flow within the 3D devices so that the results of diagnostic tests can be displayed in patterns. The patterns of the results can encode information, much like a matrix barcode, and could have numerous applications in the context of point-of-care diagnostics and home health care.

The fabrication of 3D microPADs using toner involves four steps: (i) patterning a sheet of paper, (ii) printing four layers of toner on the top face of the patterned paper waiting five minutes between each print cycle as described previously, (iii) folding the paper to generate the layered 3D device, and (iv) passing the folded paper through a thermal laminator to bond the layers of paper together (Fig. 1A). We have bonded up to eight layers of paper using this procedure. The entire process can be completed in approximately 30 min, and when multiple devices were fabricated in parallel, a single researcher was able to make 160 devices (each containing four layers of paper) in 50 min (Fig. S1).

In this work, we used wax printing to pattern Whatman 1CHR paper, though other papers and methods of patterning should also be compatible with the process. Toner is a convenient material for fabricating 3D microPADs because it can be printed directly on sheets of patterned paper in well-defined patterns with ports to allow fluids to wick between adjacent layers of paper. We showed previously that toner can be used to generate impermeable barriers on microPADs in order to enclose and protect the channels in the devices. Toner has also been used as a thermal adhesive to fabricate conventional plastic-based microfluidic devices.
A 1. Pattern paper 2. Print toner
   wax  
   paper  
   →  
   →  
   →  
   ↓
   toner  
   port  
   sample inlet
   ↓  
   →  
   →  
   →  
   →
   4. Laminate 3. Fold device

B

C

D 1 mm

port
toner
wax
paper

Fig. 1 Fabrication of 3D microPADs using toner. (A) Schematic of the four steps to fabricate a device. (B) The top of the device after adding aqueous dyes to the sample inlets and allowing them to wick across the channels for 2 min. (C) The bottom of the device after adding aqueous dyes. (D) Cross-section of the device showing the layers of paper and toner. The toner bonds the layers of paper together and prevents fluids in adjacent channels from mixing. Ports in the toner layer allow fluids to wick between adjacent layers of paper.

In our 3D microPADs, toner serves as both a thermal adhesive and an impermeable barrier.

We found that folding a sheet of patterned paper, rather than stacking pieces of patterned paper, was more convenient for aligning and assembling the layers of patterned paper in a 3D device. A strip of patterned paper can either be folded in a zigzag pattern or folded around itself to generate the layered structure. These two folding patterns allow for multiple devices patterned on a sheet of paper to all be folded and then laminated simultaneously (Fig. S1). While the thermal laminator is a convenient and inexpensive (~$30) instrument for fabricating the devices, other heat sources could be used instead, such as an oven (set to 170 °C for one minute), a hotplate or an iron. One drawback of the thermal lamination step is that it will likely damage heat-sensitive reagents, which means heat-sensitive reagents would have to be applied after the device is fully assembled.

To demonstrate some of the capabilities of these 3D devices, we prepared a variety of devices for distributing samples from four sample inlets on the top of the devices into arrays of 16, 25 or 64 test zones located on the bottom of the devices (Fig. 2). The fluids can be distributed into virtually any pattern by modifying the network of channels in the middle layers of paper in the device (Fig. S2).

One of the original motivations for developing microPADs was to produce low-cost point-of-care diagnostic devices that could be used to detect analytes in remote villages, in the field by first responders, and at home by untrained users. Potential challenges with widespread use of point-of-care diagnostics will be keeping track of the results of the assays; identifying counterfeit, expired or defective devices that may provide incorrect results; and protecting patient privacy. Three-dimensional microPADs offer a solution to all of these challenges by encoding information into the way that the results of colorimetric assays are displayed on the device and by incorporating positive and negative controls for each assay.

A device that distributes one sample into an array of 16 test zones could display the results of a colorimetric assay in any one of 65,535 unique patterns (Fig. 3). If multiple assays are performed or a larger array of test zones is used, many more patterns can be achieved. The patterns of the results can be programmed in two different ways: (i) fluid flow can be controlled by changing the patterns of channels or the toner layers in the device so that the sample is only delivered to certain test zones and (ii) the reagents for the assays can be applied only in select test zones so that only those zones generate color changes.

Patterned results could serve as a matrix barcode to encode information such as the type of assay being run, the identity of the sample, or the date when or location where the assay was performed. The patterns could be imaged and read with a camera phone, and the information could be automatically delivered to a centralized database where the results could be archived. This type of system could be useful in monitoring patient compliance with treatments. Patterned results also provide a simple system for identifying counterfeit devices. For example, each pattern of results could be associated with a unique code that could be printed on the device. After running the assay, the user would photograph the results using a camera phone and enter the code on the device. If the pattern and the code did not match, the user would know that the device was counterfeit, and the results would be discarded.

To identify defective or expired devices, known amounts of analyte can be stored on the middle layers of paper in a device so that positive control assays can be run simultaneously with the sample, and no additional samples or fluid addition steps would be required to operate the device (Fig. 3G and H). To demonstrate,
we added a 100 mM glucose solution to specific locations on a device and allowed it to dry before the device was folded and laminated (Fig. S3). After the device was assembled, reagents for a glucose assay were spotted in select test zones on the bottom of the device to form the desired pattern. The test zones that did not contain reagents served as negative controls. When the sample was added to the sample inlet, it wicked through the channels dissolving the dried glucose and carrying it into the test zones where a color change occurred in the positive control zones even if no glucose was present in the sample. If the device was defective, or the reagents were no longer active, the positive control test zones would not produce the expected color change, or a color change might be observed in a negative control test zone, and the results from the device would be discarded. If the device functioned properly, the controls could be useful for calibrating
and quantifying the results. The limitations to incorporating positive controls into a device are that the target analyte must survive the thermal lamination step, it must dissolve into the sample as the sample wicks through the device, and it must be stable for at least the same amount of time as the reagents for the assay.

Devices that incorporate positive and negative controls could also be useful for encrypting the results of a diagnostic test to protect patient privacy. If multiple positive control spots with different concentrations of analyte were incorporated into a device, the person running the test would not know which test zones corresponded to positive controls, negative controls and patient results. Only after analyzing the device using a camera phone or other instrument and, for example, entering a code supplied with the device, would the results of the test be accessible.

In this paper, we present a new and simplified method for preparing 3D microPADs using toner and introduce the concept of patterned assay results. The method of fabrication can be readily adopted by researchers since virtually all research laboratories already have access to a laser printer and some type of heat source. By programming the configuration of the results of colorimetric assays into patterns and by incorporating positive and negative controls into a device, many of the challenges of point-of-care diagnostic testing can be overcome.

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References

12. Calculated from $2^{16} – 1 = 65,535$. We subtracted one because the device must display at least one positive result.