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# New Functionalities for Paper-Based Sensors Lead to Simplified User Operation, Lower Limits of Detection, and New Applications

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## Keywords

paper analytical device, point-of-care, microfluidic device

## Abstract

In the last decade, paper analytical devices (PADs) have evolved into sophisticated yet simple sensors with biological and environmental applications in the developed and developing world. The focus of this review is the technological improvements that have over the past five years increased the applicability of PADs to real-world problems. Specifically, this review reports on advances in sample processing, fluid flow control, signal amplification, and component integration. Throughout, we have sought to emphasize advances that retain the main virtues of PADs: low cost, portability, and simplicity.

## 1. INTRODUCTION

The objective of the present review is to provide a snapshot of the field of paper analytical devices (PADs), particularly the thought processes that have led us to the current state of the art. The advances in PADs we discuss here have made it possible to carry out increasingly sophisticated assays using inexpensive paper platforms. Over the last five years, a number of enabling technological advances have been introduced to paper devices, including timed delivery of reagents (1–3), magnetic concentration (4–7), signal amplification (4, 8–11), automatic on-device washing (1, 2, 10), hollow channels (4, 12–14), and blood separation (15–17). The powerful yet simple PAD shown in **Figure 1**, known as the *No*Slip (discussed in Section 7), is an example of a paper device that integrates several of these individual advances for detection of proteins in the low picomolar range (14). Although many of the technologies discussed in this review are common in more sophisticated assay systems, some clever science and engineering has been required to introduce them into simple paper devices costing less than US$2, which is a benchmark for low-cost point-of-care applications.

The PAD field is moving very quickly, and the specific advances we discuss here will likely be superseded by even more clever and functional systems within just a few years. We hope, however, that the general principles we discuss will provide guidance to future researchers who will develop the tools necessary for PADs to become a routine part of health care systems worldwide.

The PADs discussed in this review were developed within the last five years and can perform quantitative assays using a handheld reader such as a camera phone, portable potentiostat, digital multimeter, commercial glucose meter, or stopwatch. Specifically excluded from this article are

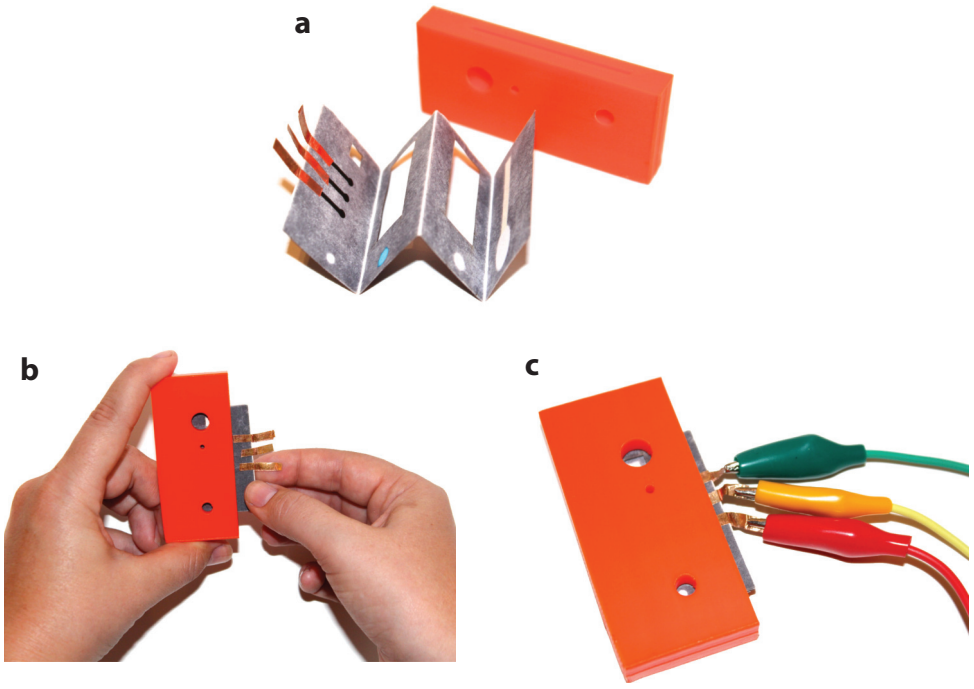


**Figure 1**

Photographs of a sophisticated electrochemical paper analytical device (PAD) known as the *No*Slip. (*a*) The *No*Slip and its 3D-printed holder. (*b*) The *No*Slip being inserted into its holder. (*c*) The fully assembled PAD connected to an electrochemical measurement device (not pictured).

important closely related topics for which excellent recent reviews already exist. These include the history (18–21; see also 22, 23 for more information on lateral flow assays) and fabrication (19, 20, 24–28) of PADs as well as the various detection methods (18, 22, 24, 27, 29) used by PADs. In addition, several very fine recent reviews contain subject matter that to some degree overlaps with that discussed here. These include a comprehensive review about the fabrication and applications of chemical sensing PADs by Henry and coworkers (28) and a perspective on recent technological and engineering advances by Abbas and coworkers (30). The specific focus here on improved device functionality, underpinned by increasingly sophisticated design concepts that are intended to simplify the operation of PADs by end users, distinguishes the present review from earlier reports.

## 2. MULTIDIMENSIONAL PAPER DEVICES

Multidimensionality in paper devices enables multiplexing, reduces size, and incorporates additional sample processing functions. For example, the invention of two-dimensional (2D) PADs by Whitesides and coworkers (31) made it possible to carry out separations (15, 16, 32–34) and simultaneously detect multiple analytes using a single sample reservoir (11, 35–44). The subsequent development of three-dimensional (3D) PADs led to even more sophisticated operations, such as on-device delivery of reagents (4, 8, 45–49), controlled flow rates (45, 47, 50–52), fluidic switches (3, 53–55), and the ability to incorporate microscale objects into assays (4, 7, 45). Numerous publications have focused on the basic operating principles and fabrication methods for 2D and 3D PADs (56, 57), and therefore they will be described only briefly here.

### 2.1. Two-Dimensional Paper Analytical Devices

Two-dimensional PADs date back to 1949, when Müller & Clegg (58) used an embossing tool to form patterns of hydrophobic and hydrophilic domains on a piece of paper. However, Whitesides and coworkers (31) ushered in the modern era of 2D PADs in 2007. 2D PADs are easily fabricated by patterning a single piece of paper (often chromatography paper) with a hydrophobic material, such as photoresist or wax, to define hydrophilic reservoirs and microfluidic channels. Fluid flow is usually driven by capillary forces present within the hydrophilic, fiber-containing regions of the PAD. Often, assay-specific reagents are spotted onto predefined regions of the paper after patterning (2, 40). For real-world applications, some sort of device packaging is also provided to make the PAD functional in the hands of a user (33, 59–62).

A wide variety of detection modalities have been applied to 2D PADs. For example, colorimetric assays have been developed to test for amino acids (63), bacteria (35, 64), biomarkers for cancer (11), lung (36) and liver (17, 65) function, gases (66–68), infectious diseases (69), ions (37, 38, 70–72), heavy metals (39–41, 73–80), small molecules (42, 72, 74, 81–83), pH (37, 44, 84), and proteins (1, 85, 86). In all of these cases, complexing agents, nanoparticles, or enzymes were used to generate a visible color change. Electrochemical methods have also been used to detect a wide range of targets on 2D PADs, including antibiotics (87), gases (88, 89), metals (90), pH (91), and proteins (10, 92). Physical parameters, such as force (93), infrared (94) and UV (95) light, strain (96), and temperature and humidity (97), have also been detected electrochemically. A number of specific electrochemical techniques have been adapted to 2D PADs, including relative resistivity, impedance, amperometry, conductivity, and voltammetry (linear, cyclic, square wave, and differential pulse).

In the sections below, we highlight two specific examples of 2D PADs that, taken together, typify some of the interesting advances that are characteristic of the field. The first is a 2D PAD

for the detection of a protein marker for malaria, developed by Yager and coworkers (98); the second is a device for the detection of glucose, developed by Laiwattanapaisal and coworkers (32).

## 2.2. Three-Dimensional Paper Analytical Devices

The fabrication of 3D PADs involves additional complexities that need not be considered for 2D devices. Foremost among these challenges is the requirement for fluidic communication between layers of the device, but providing for even compression of the layers and more complex packaging are also important.

There are two common methods for fabricating 3D PADs. The first was devised by Whitesides and coworkers (57) in 2008 and uses multiple pieces of wax-patterned paper that are aligned and stacked upon one another. This important advance stimulated others to think about constructing PADs in 3D, but the fabrication methodology used in this first publication is complex and does not lend itself to low-cost manufacturing due to the inherent difficulty of aligning and affixing the individual layers. The second method, first developed by our group in 2011 (56), is much simpler, resolves the problems of cost, and provides additional benefits. In this case, a single piece of wax-patterned paper is folded using the principles of origami, such that the pre-patterned channels self-align and the vias (required for vertical fluid flow) are in fluidic contact (56). Moreover, because the individual layers are not taped together, the device can be easily unfolded to read out assay results within its interior layers. We call this family of devices *o*PADs, because they are assembled by origami. An example of an early-stage *o*PAD is shown in **Figure 2**.

The three most prominent detection strategies used for 3D PADs are colorimetry, electrochemistry, and time-based measurements. For colorimetry using 3D PADs, assays have been described for bacteria (99), a virus (100), metals (79, 101, 102), small molecules (5, 32, 50, 103–105), anions (106), and proteins (46, 50, 56, 104). Electrochemistry has been used to detect targets including DNA (6, 62, 107), heavy metals (101, 108), small molecules (45, 109), gases (110), ions (111, 112), and proteins (7, 9, 60, 61, 113, 114). Time-based measurements have been demonstrated for enzymes (8) and small molecules (8, 47, 108, 115).

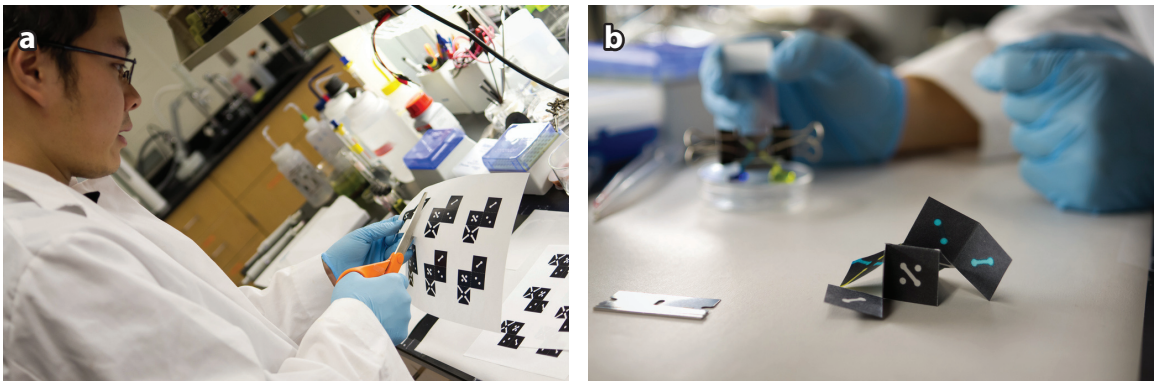


**Figure 2**

Photographic illustration of the origami fabrication process. (*a*) A sheet of chromatography paper is wax-patterned with the multiple origami paper analytical device (*o*PAD) designs. Each individual *o*PAD device is cut from the sheet and briefly heated so that the wax penetrates the thickness of the paper; the paper is then folded, compressed, and used for detection. (*b*) A partially unfolded *o*PAD that (prior to unfolding) had different colored dyes added to its inlets for demonstration purposes.

### 2.3. Hybrid Paper Analytical Devices

Each of the materials commonly used for constructing microfluidic devices (paper, glass, and plastic) has advantages and disadvantages, and the degree to which they are used depends on the intended application. For paper devices, the primary advantages are ease of fabrication, low cost, and low power requirements (a pump is usually not required). Other materials provide more controllable flow characteristics, smaller and more precise channel dimensions, better control over surface properties, transparency (for optical detection), and rigidity. Accordingly, for some paper fluidic applications, there may be advantages associated with introducing a second or third material. We consider here only those hybrid devices in which paper is the dominant or functional material. The most common paper-based hybrids are those that combine paper with either polydimethylsiloxane (PDMS) or a hard plastic.

**2.3.1. Paper and polydimethylsiloxane.** The combination of paper and PDMS can be advantageous for incubations spanning multiple hours. Paper-based spot arrays cost less and are more biodegradable than plastic 96-well plates (116); however, wax-patterned chromatography paper loses some of its structural integrity and fluid confinement ability when in the presence of aqueous solutions for periods of more than approximately 20 min. To resolve this problem, Peltonen and coworkers (117) infused a cellulose matrix with PDMS to create microarrays that can hold a larger volume of liquid for at least 50 min. After 50 min, evaporation becomes a problem. To address this, Funes-Huacca and coworkers (118) used paper as a portable, self-contained culture chamber and added a PDMS lid to supply the bacteria with oxygen while simultaneously preventing evaporation of the growth medium. Clearly there are some advantages of combining paper and PDMS, but of course those advantages must outweigh the additional cost and complexity of hybrid device fabrication.

**2.3.2. Paper and plastic.** The integration of plastic into PADs has been shown to provide additional functionality including sophisticated valving (53), reduced rates of evaporation (119), and enhanced rigidity (59, 119). For example, Lutz and coworkers (33) combined paper and plastic for multiplexed detection of a malaria antigen and a salmonella antibody in patient plasma samples. In this case, the plastic reduced the evaporation rate and increased durability, whereas the paper regulated flow, stored predried reagents, metered fluid volume, filtered the sample, and acted as a batch mixer (see Section 4.5).

For electrochemical detection, carbon ink electrodes printed on PADs using a stencil (4, 7, 120) or screen (108, 121) are satisfactory for most applications. However, the porous nature of chromatography paper can lead to electrodes that are nonuniform, insufficiently conductive, or not robust enough for particular applications. Electrodes printed on plastic can resolve these problems (34, 61, 122), are commercially available, and have been incorporated into PADs (32, 123–126).

## 3. HOLLOW CHANNELS

The cellulose fiber network usually present in PAD channels (we refer to these as paper channels) can be advantageous or disadvantageous depending upon the application. There are three common problems with paper channels. First, the mesh size in typical paper networks (~10 μm) does not allow unhindered flow of micrometer-scale objects, such as microbeads or bacteria (127). Second, the high surface area of cellulose fibers can lead to a significant degree of nonspecific absorption. Third, the rate of fluid flow through paper is retarded by the presence of the cellulose fibers (50).

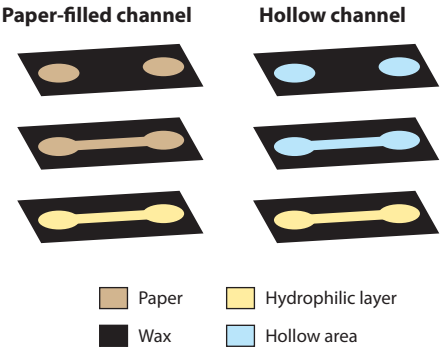


**Figure 3**

Comparison of paper-filled and hollow channels. Wax-patterned boundaries define both types of channels. The difference is that the cellulose matrix has been removed from the hollow channel. The bottom layer of the hollow channel is rendered hydrophilic so that fluid can flow by capillary action. Figure adapted with permission from Reference 50.

All three of these deficiencies can be resolved by simply removing the paper within a channel (**Figure 3**).

The fabrication of hollow channels is very simple and involves patterning a paper substrate with wax to define the channel network and then removing the cellulose content from the channels using a razor blade or laser cutter. To ensure capillary flow, however, it is also necessary to provide a hydrophilic wall within the channel (50). If all four walls are impregnated with wax or another hydrophobic substance, then a pump is required for fluid flow (128).

We recently developed specific methods for creating hollow channels and also described some of their characteristics (13, 50). For example, their flow rate can be controlled by balancing capillary and pressure forces (50). Indeed, the pressure resulting from even a single drop of liquid (~10 μL) is sufficient to induce fast, laminar flow through hollow channels. Moreover, the flow rate in hollow channels is typically about sevenfold higher than in conventional paper channels (50). Researchers have taken advantage of hollow or open channels to detect small molecules (12, 13, 50), metal ions (102), the toxin ricin (7), DNA (6), and bovine serum albumin (50).

## 4. CONTROLLED FLUID ACTUATION AND MANIPULATION

There are numerous detection strategies that require sequential addition of reagents, timed incubation, mixing, washing, or a combination thereof. These are, of course, routine operations for large-scale laboratory instruments and even for plastic and glass microfluidic devices, but until just a few years ago they were unachievable in PADs. In this section, we describe recent approaches for overcoming the obstacles associated with integrating these types of sophisticated operations into simple paper devices.

### 4.1. Slipping

Glass slip chips were first developed in 2010 by Ismagilov and coworkers (129), and in 2013 our group introduced analogous paper devices called SlipPADs (54). Later, Pollock and colleagues (55) reported an essentially identical device called a Paper Machine. The latter two innovations achieve controlled fluid manipulation, including timed delivery of reagents and timed incubation,

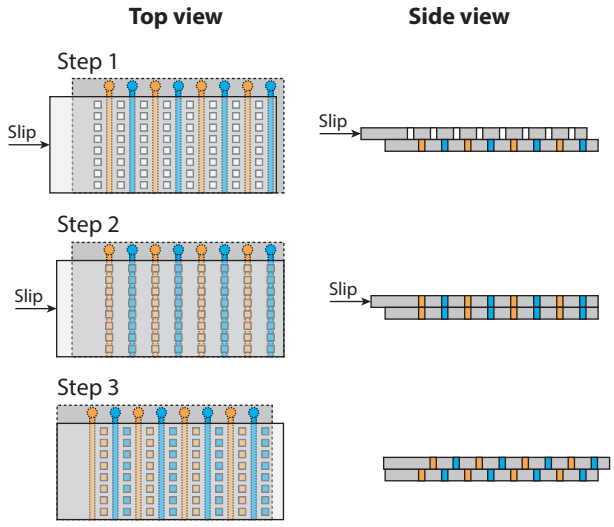


**Figure 4**

Schematic illustrations outlining the operation of a slipping paper analytical device (SlipPAD) for parallel fluid manipulation. This SlipPAD consists of two wax-patterned layers. Black dashed lines in the top views highlight the bottom layers and their patterned fluidic channels and reservoirs. Figure reprinted with permission from Reference 54.

by slipping one sheet of wax-patterned paper into alignment with another. The SlipPAD is particularly versatile in this regard, because it can be used for high-throughput parallel reactions or for sequential addition of multiple reagents.

**Figure 4** is a schematic illustration showing the operation of a simple SlipPAD our group devised for generating calibration curves and performing concurrent assays. In Step 1, colored reagents are loaded into defined channels, and then in Step 2 the slip layer is moved so that an array of 285 paper wells is simultaneously loaded with the reagent. In Step 3, the paper wells are isolated from the filling channels. The SlipPAD approach has also enabled controlled movement of reagents at the time of need in biosensing applications (4, 6, 7, 107).

## 4.2. Channeling

The strategic arrangement of paper channels provides a means for controlled delivery of pre-dried reagents and increased assay automation on PADs (1, 2, 33, 98, 130–132). In the case of **Figure 5**, for example, channel length controls the timing of reagent delivery to the reaction zone for a malaria assay. Specifically, detection antibodies labeled with gold nanoparticles, washing buffer, and gold enhancement solution were predried on the individual timing channels. By simultaneously applying fixed volumes of sample to each of the three fluid application zones, the predried reagents were rehydrated and delivered by fluidic wicking to the test and control lines. In **Figure 5**, the contents of the shortest (far right) channel (containing gold-labeled detection antibodies) were first to reach the test and control lines, followed by the wash solution from the middle channel and the gold enhancement solution from the far left channel. This judicious arrangement of channels is advantageous, because it provides a simple way to complete multiple assay steps with minimal user intervention.

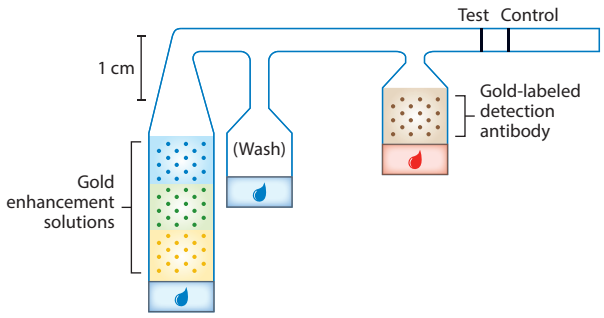


**Figure 5**

Schematic illustration of a 2D paper network for assaying *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2). Text labels and colored dots indicate the location of each reagent. Controlled delivery of reagents and wash buffer to the detection zone is achieved by varying the channel length. The red drop indicates the addition of the sample, and the blue drops represent buffer. Figure reprinted with permission from Reference 1.

## 4.3. Delaying

An alternative method for timed delivery of reagents on PADs is the use of a fluidic delay, such as a one-way fluidic delay switch (51), shunt (133), or dissolvable reagent (134), that slows or temporarily inhibits capillary flow. For example, Fu and coworkers (133) integrated an absorbent sink into a PAD channel with the intent of slowing capillary flow by absorbing some of the fluid. Additionally, Phillips and coworkers (8, 47, 52) developed a novel detection strategy, wherein an analyte converts a predried hydrophobic blocking material in the channel to a hydrophilic form that facilitates flow. The analyte concentration is either directly or inversely proportional to the additional time required for the analyte to flow from the inlet to the outlet in comparison to a reference channel.

## 4.4. Switching

The manipulation of flow in 3D PADs can also be achieved using a fluidic switch that initiates flow on demand. The switching function can be achieved by using a fluidic diode (135), a physically moveable paper flap that acts as a valve (38), or a press-and-flow button (3). For example, the press-and-flow button (**Figure 6**) can be pressed to initiate flow at the discretion of the user. Specifically, in this example, a stylus is used to press the single-use "on" button, which activates a connection between two paper channels that were previously separated by a hollow, hydrophobic gap. Whitesides and coworkers (3) initiated flow with a single-use "on" switch to detect glucose, proteins, ketones, and nitrite in artificial urine.

## 4.5. Mixing

Fluid mixing is an important operation for many types of assays. This is because flow in hollow and paper channels is generally laminar (50, 136). Accordingly, mixing of fluidic streams occurs only by diffusion and is, therefore, slow. Only two examples have thus far been published to address the need for mixing. In one case, Yager and coworkers (33) demonstrated a batch mixer on an immunoassay card by using an air permeable vent that allowed air bubbles into the mixing chamber to induce convection. Yeo and coworkers (137) accomplished uniform mixing on a PAD using surface acoustic wave energy. There is clearly a need for more work in this area.

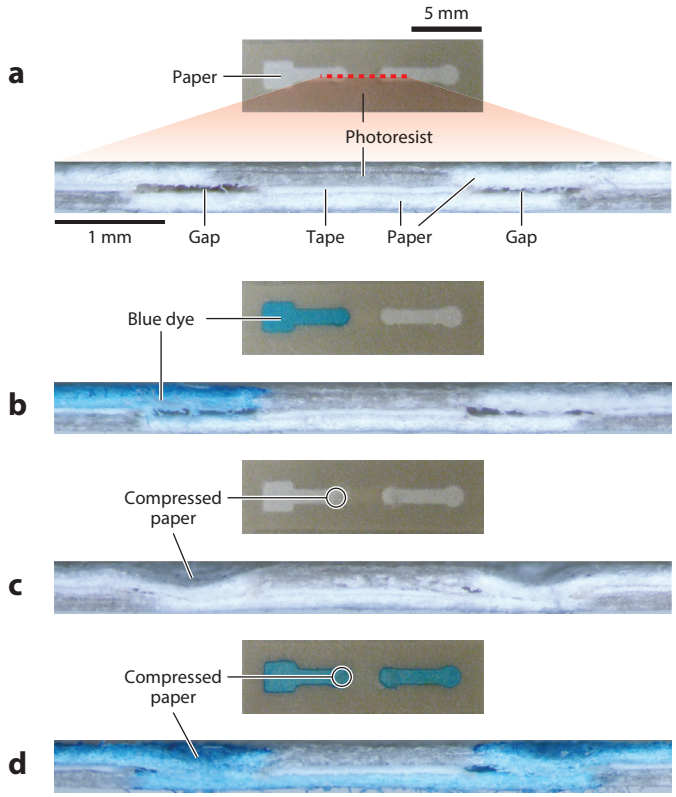


**Figure 6**

Top view and cross sections of a channel containing a fluidic "on" button for use with a programmable paper analytical device constructed of paper and tape. (*a*) A fully assembled device; the cross-sectional view is obtained by cutting the device along the dashed line in the top view. (*b*) The device shown in panel *a* after adding 10 μL of aqueous blue dye to the left side of the channel. (*c*) The device in panel *b* after compressing the top layer of paper with a ballpoint pen to connect the gap. (*d*) The device shown in panel *c* after the dye has flowed past the fluidic "on" button. Figure reprinted with permission from Reference 3.

## 5. NANO- AND MICROSIZED OBJECTS

Nano- and microsized objects can improve analyte detection in PADs in several important ways. First, recognition agents, such as antibodies or aptamers, can be anchored to mobile particle surfaces, leading to a high number of recognition elements compared to planar surfaces. Accordingly, these nano- and microsized surfaces provide higher binding efficiencies for solution-phase targets due to their increased binding capacity (138). Second, the speed of target binding can be enhanced on particles, compared to stationary surfaces, due to their mobility. Third, particles can be used to amplify detection signals on PADs, thereby eliminating the need for enzymatic amplification.

Over the past few years, there have been many reports of the use of nanoparticles for colorimetric (1, 33, 35, 39, 63, 69, 74, 75, 79, 81, 84, 139–141) and electrochemical (4–7, 10, 35, 51, 61, 62, 102, 142, 143) detection on both 2D and 3D PADs. Nanotubes, -rods, or -wires comprised of carbon (60, 61, 87–89, 91, 102, 111, 113), zinc oxide (96), gold (86, 142), platinum (144), nickel (144), or copper (144) have been integrated into paper-based sensors to enhance detection. Microsized objects that have been used with PADs include magnetic and nonmagnetic microbeads (45) and microcapsules (145, 146).

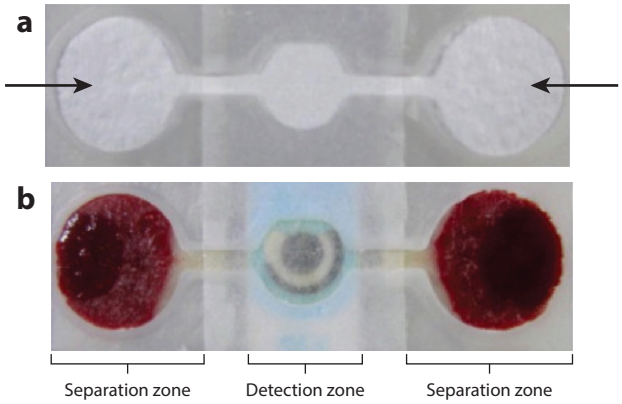


**Figure 7**

Top-view photographs of a dumbbell-shaped paper analytical device (PAD) for whole blood separation. (*a*) The device prior to the addition of blood; the arrows indicate the location of blood filters and the direction of flow. (*b*) The PAD after the addition of blood. The filters exclude the red blood cells while the plasma travels to the electrochemical detection zone at the center. Figure reprinted with permission from Reference 32.

## 6. FILTRATION AND SEPARATION

The properties of chromatography paper provide a natural avenue for filtration and separation on PADs, as has been demonstrated in experiments involving molecules (34, 147) and viruses (100). However, chromatography paper is not effective for removing interferences, such as blood cells. Therefore, detection in blood samples on PADs requires a separation membrane (15–17, 32, 33, 49, 65). For example, a dumbbell-shaped electrochemical PAD (**Figure 7**) quantitatively assayed glucose in blood plasma that was isolated from whole blood using a membrane (32). In **Figure 7*a***, the separation zones contain blood separation membranes, whereas the detection zone contains chromatography paper overlaid onto a three-electrode electrochemical cell. The operation of the device involves depositing a sample of whole blood onto the two separation zones (**Figure 7*b***). The membranes trap the red and white blood cells while the blood plasma continues to flow toward the detection zone. The symmetrical movement of blood plasma toward the central reservoir ensures uniform flow over the electrodes. In this PAD, glucose was detected amperometrically using a commercial Prussian blue–modified, screen-printed carbon working electrode that measured the amount of peroxide resulting from the reaction between glucose and glucose oxidase.

More highly resolved separations on PADs can be achieved using electrophoresis (148, 149). For example, our group demonstrated the separation of proteins using a low-voltage *o*PAD electrophoretic device (*o*PAD-Ep) (148). The separation channel in the *o*PAD-Ep was constructed by paper folding (origami) and was specifically designed to require only low voltages. We anticipate that active separation components, such as the *o*PAD-Ep, will eventually be integrated into more complex, multifunctional PAD designs in the future.

## 7. EXTRACTION AND CONCENTRATION

Additional sample processing is often required when sensing in realistic sample matrices, such as blood, serum, natural waters, or urine. Incorporating extraction or preconcentration functions into PADs can lead to new applications, lower limits of detection (LODs), shortened assay times,

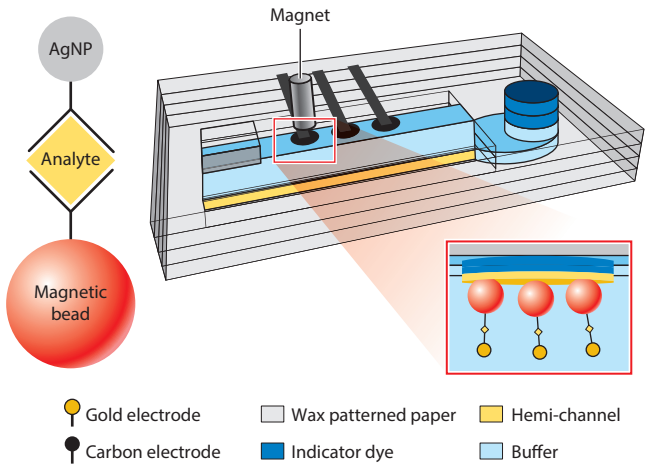


**Figure 8**

A 3D illustration of a *No*Slip device. The magnetic microbead–supported antibody sandwich (*left*) is injected into the *No*Slip (*right*). The magnetic microbeads are concentrated at the detector electrode via a magnet (*inset*), and the silver nanoparticle labels amplify the electrochemical signal.

removal of matrix effects, and device autonomy. An impressive literature focused on sample processing has emerged within the last few years, addressing enrichment (150), extraction (48, 136), preconcentration (75, 151–154), and magnetic concentration (4). For example, our group reported on a PAD (known as the *No*Slip) that utilizes magnetic concentration of magnetic microbeads (**Figure 8**) within a hollow channel (14). Specifically, a magnet is positioned directly above the working electrode, allowing for preconcentration of magnetic microbeads functionalized with specific capture agents. In the presence of the target, antibody-functionalized silver nanoparticle labels are co-located with the microbeads at the electrode. This provides a convenient means for detection and amplification of the target. In addition to concentrating the silver nanoparticle labels at the electrode, subsequent anodic stripping voltammetry provides a second level of concentration. These sample concentration steps lead to a LOD of 2.1 pM for a model complex in a urine matrix (14).

## 8. AMPLIFICATION AND ENHANCEMENT

By incorporating amplification and signal enhancement into PADs, lower LODs, tunable dynamic ranges, and higher sensitivities can be achieved within shorter timeframes. Signal amplification has been demonstrated on PADs through the use of enzymes, nanoparticles, capacitors, and polymerization, all of which are described in this section.

### 8.1. Enzymes

The most common method of signal amplification involves the use of enzymes, which act on a substrate molecule to produce a color change or electrochemically active redox molecule. With colorimetric readout, enzymes have been used on PADs to detect antibodies (116), bacteria (64), cells (99), heavy metals (77), small molecules (including sugars, acids, oxons, and organics) (42, 72, 82, 86, 105, 141, 155), and proteins (17, 86). There has also been some work on improving the long-term stability of enzymes stored on PADs (145, 146). Enzymes have also been used on

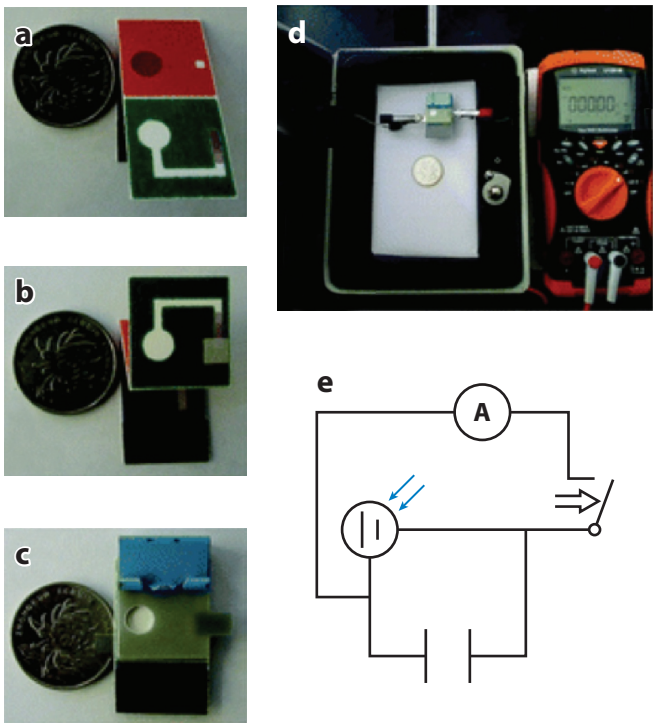


**Figure 9**

(*a–d*) Photographs of an origami paper analytical device outfitted with an on-device supercapacitor for signal amplification. (*a*,*b*) The device is constructed by origami folding. (*c*) The folded device is clamped to a circuit board with a binder clip. (*d*) The clamped device is placed into a cassette and connected to a digital multimeter. (*e*) Circuit diagram of the electronic setup. Figure adapted with permission from Reference 51.

PADs for electrochemical detection of biomarkers (9–11, 60, 61, 113), heavy metals (108), and small molecules (including sugars and toxins) (5, 108).

## 8.2. Nanoparticles

A particularly advantageous amplification approach involves the use of metal nanoparticles as charge carriers (4) or catalysts (1, 98, 131) for electrolytic or electroless deposition, respectively. In comparison to enzymes, there are two main advantages to using nanoparticles for amplification. First, there are many straightforward methods known for immobilizing recognition elements (e.g., antibodies or DNA) onto nanoparticles. Second, nanoparticles do not require long reaction times or have limited stability, which are common problems with enzymes. As discussed in the previous section, our group has shown that silver nanoparticles can be detected on PADs in the high femtomolar (4) to low picomolar (7, 14) range, which is well matched to many biomarkers.

## 8.3. Capacitors

A capacitor can be coupled with a PAD fluidic network to amplify electronic signals by storing and then releasing charge to achieve gain (35, 45, 51, 156). For example, Huang and coworkers (51) fabricated a capacitor by drawing thin film graphite electrodes with a pencil and dipping

the electrodes in a sulfuric acid and polyvinyl alcohol gel electrolyte. **Figure 9** demonstrates the origami folding (**Figure 9*a–b***) and compression (**Figure 9*c***) of the device, along with the cassette into which the *o*PAD is inserted (**Figure 9*d***). An on-device fluidic delay switch controls the actuation of the capacitor (**Figure 9*e***). In the presence of target DNA, chemically modified gold nanoparticle labels participate in a photoelectrochemical process that results in the supercapacitor being charged. The PAD was able to detect DNA at low femtomolar concentrations by utilizing a sandwich assay.

### 8.4. Polymerization and Depolymerization

Kang and coworkers (11) showed that a polymerization reaction could be used to significantly amplify a binding event on a 2D PAD. Specifically, target capture triggered a radical polymerization reaction, and the resulting polymer provided multiple binding sites to enhance the signal resulting from a standard electrochemical enzyme-linked immunosorbent assay (ELISA). The ELISA was carried out by sequential addition of antibodies, a blocking protein, and polymerization reagent, with thorough washing between each addition. Using this method, an LOD of 10 pg/mL was achieved for detection of cancer biomarkers.

Depolymerization has also been used to amplify signals. For example, Phillips and coworkers showed that an enzyme target could be detected in the low- to mid-femtomolar range using very clever chemistry and a 3D PAD (8). The target concentration was determined by measuring the time required for depolymerization of a hydrophobic gate arising from the presence of peroxide generated during detection.

## 9. CONCLUSIONS AND OUTLOOK

The movement toward smarter paper platforms outfitted with all device components necessary for sophisticated sensing chores is quickly becoming a reality. Goals for the coming years include further simplification of design and construction, integration of new functions such as separations and preconcentration, and minimization of user intervention. At the present time, a number of specific problems require attention. These include adding on-board storage of reagents and ensuring their stability; figuring out how to efficiently resolvate those reagents at the time of need; eliminating nonspecific adsorption; and purifying matrices, such as blood, prior to analysis. All of this needs to be accomplished without introducing too much cost, or the advantage of using paper as a platform is lost.

A few other important points not discussed in detail thus far should be mentioned. If paper sensors are to be used in the developing world or by the lay public in the developed world, then either visual readout or cheap, foolproof electronic readers are required. The obvious choice for the latter is cell phones, but how exactly these will be connected to the sensors, how the sensors will be powered, and how wireless communication of results will be secured remain open questions.

At this stage in their development, it is difficult to say what commercial paper-based sensors are going to look like in four or five years. It is certain, though, that individuals will need to take more control over their own health care needs in the coming years. Home management of chronic disease is a good example. In the United States, this is, in part, a consequence of the Affordable Care Act, which has added millions of newly insured people to the health care system without a corresponding increase in the number of doctors. In many parts of the developing world, national health care systems either do not exist or are so underfunded that they might as well not exist. In both cases, the ability of an individual to routinely check his or her biomarkers and transmit the results wirelessly to distant clinics will be immensely valuable.

Two final points should be mentioned. First, inexpensive personal diagnostic devices will be able to provide early warning of massive contagions, such as the Ebola outbreak of 2014, as well as information about how such diseases spread. Second, lifetime testing by individuals, which provides personal baselines for key biomarkers, may lead to early detection of cancer and other deadly diseases.

## DISCLOSURE STATEMENT

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## LITERATURE CITED


1. Fridley GE, Le H, Yager P. 2014. Highly sensitive immunoassay based on controlled rehydration of patterned reagents in a 2-dimensional paper network. *Anal. Chem.* 86(13):6447–53
2. Apilux A, Ukita Y, Chikae M, Chailapakul O, Takamura Y. 2013. Development of automated paper-based devices for sequential multistep sandwich enzyme-linked immunosorbent assays using inkjet printing. *Lab Chip* 13(1):126–35
3. Martinez AW, Phillips ST, Nie Z, Cheng C-M, Carrilho E, et al. 2010. Programmable diagnostic devices made from paper and tape. *Lab Chip* 10(19):2499–504
4. Scida K, Cunningham JC, Renault C, Richards I, Crooks RM. 2014. Simple, sensitive, and quantitative electrochemical detection method for paper analytical devices. *Anal. Chem.* 86(13):6501–7
5. Ge S, Liu W, Ge L, Yan M, Yan J, et al. 2013. In situ assembly of porous Au-paper electrode and functionalization of magnetic silica nanoparticles with HRP via click chemistry for Microcystin-LR immunoassay. *Biosens. Bioelectron.* 49:111–17
6. Li X, Scida K, Crooks RM. 2015. Detection of hepatitis B virus DNA with a paper electrochemical sensor. *Anal. Chem.* 87(17):9009–15
7. Cunningham JC, Scida K, Kogan MR, Wang B, Ellington AD, Crooks RM. 2015. Paper diagnostic device for quantitative electrochemical detection of ricin at picomolar levels. *Lab Chip* 15(18):3707–15
8. Lewis GG, Robbins JS, Phillips ST. 2013. Point-of-care assay platform for quantifying active enzymes to femtomolar levels using measurements of time as the readout. *Anal. Chem.* 85(21):10432–39
9. Li L, Xu J, Zheng X, Ma C, Song X, et al. 2014. Growth of gold-manganese oxide nanostructures on a 3D origami device for glucose-oxidase label based electrochemical immunosensor. *Biosens. Bioelectron.* 61:76–82
10. Wu Y, Xue P, Kang Y, Hui KM. 2013. Paper-based microfluidic electrochemical immunodevice integrated with nanobioprobes onto graphene film for ultrasensitive multiplexed detection of cancer biomarkers. *Anal. Chem.* 85(18):8661–68
11. Wu Y, Xue P, Hui KM, Kang Y. 2014. A paper-based microfluidic electrochemical immunodevice integrated with amplification-by-polymerization for the ultrasensitive multiplexed detection of cancer biomarkers. *Biosens. Bioelectron.* 52:180–87
12. Fosdick SE, Anderson MJ, Renault C, DeGregory PR, Loussaert JA, Crooks RM. 2014. Wire, mesh, and fiber electrodes for paper-based electroanalytical devices. *Anal. Chem.* 86(7):3659–66
13. Renault C, Anderson MJ, Crooks RM. 2014. Electrochemistry in hollow-channel paper analytical devices. *J. Am. Chem. Soc.* 136(12):4616–23
14. Cunningham JC, Kogan MR, Tsai Y-J, Luo L, Richards I, Crooks RM. 2015. Paper-based sensor for electrochemical detection of silver nanoparticle labels by galvanic exchange. *ACS Sens.* 1(1):40–47
15. Songjaroen T, Dungchai W, Chailapakul O, Henry CS, Laiwattanapaisal W. 2012. Blood separation on microfluidic paper-based analytical devices. *Lab Chip* 12(18):3392–98
16. Yang X, Forouzan O, Brown TP, Shevkoplyas SS. 2012. Integrated separation of blood plasma from whole blood for microfluidic paper-based analytical devices. *Lab Chip* 12(2):274–80

17. Vella SJ, Beattie P, Cademartiri R, Laromaine A, Martinez AW, et al. 2012. Measuring markers of liver function using a micropatterned paper device designed for blood from a fingerstick. *Anal. Chem.* 84(6):2883–91
18. Nery E, Kubota L. 2013. Sensing approaches on paper-based devices: a review. *Anal. Bioanal. Chem.* 405(24):7573–95
19. Martinez AW, Phillips ST, Whitesides GM, Carrilho E. 2009. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal. Chem.* 82(1):3–10
20. Jeong S-G, Kim J, Nam J-O, Song YS, Lee C-S. 2013. Paper-based analytical device for quantitative urinalysis. *Int. Neurourol. J.* 17(4):155–61
21. Then WL, Garnier G. 2013. Paper diagnostics in biomedicine. *Rev. Anal. Chem.* 32(4):269–94
22. Yetisen AK, Akram MS, Lowe CR. 2013. Paper-based microfluidic point-of-care diagnostic devices. *Lab Chip* 13(12):2210–51
23. Chin CD, Linder V, Sia SK. 2012. Commercialization of microfluidic point-of-care diagnostic devices. *Lab Chip* 12(12):2118–34
24. Liana DD, Raguse B, Gooding JJ, Chow E. 2012. Recent advances in paper-based sensors. *Sensors* 12(9):11505–26
25. Mace C, Deraney R. 2014. Manufacturing prototypes for paper-based diagnostic devices. *Microfluid. Nanofluid.* 16(5):801–9
26. Rozand C. 2014. Paper-based analytical devices for point-of-care infectious disease testing. *Eur. J. Clin. Microbiol. Infect. Dis.* 33(2):147–56
27. Li X, Ballerini DR, Shen W. 2012. A perspective on paper-based microfluidics: current status and future trends. *Biomicrofluidics* 6(1):011301
28. Cate DM, Adkins JA, Mettakoonpitak J, Henry CS. 2015. Recent developments in paper-based microfluidic devices. *Anal. Chem.* 87(1):19–41
29. Maxwell EJ, Mazzeo AD, Whitesides GM. 2013. Paper-based electroanalytical devices for accessible diagnostic testing. *MRS Bull.* 38(4):309–14
30. Ahmed S, Bui M-PN, Abbas A. 2016. Paper-based chemical and biological sensors: engineering aspects. *Biosens. Bioelectron.* 77:249–63
31. Martinez AW, Phillips ST, Butte MJ, Whitesides GM. 2007. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew. Chem. Int. Ed.* 46(8):1318–20
32. Noiphung J, Songjaroen T, Dungchai W, Henry CS, Chailapakul O, Laiwattanapaisal W. 2013. Electrochemical detection of glucose from whole blood using paper-based microfluidic devices. *Anal. Chim. Acta* 788:39–45
33. Lafleur L, Stevens D, McKenzie K, Ramachandran S, Spicar-Mihalic P, et al. 2012. Progress toward multiplexed sample-to-result detection in low resource settings using microfluidic immunoassay cards. *Lab Chip* 12(6):1119–27
34. Carvalhal RF, Simão Kfouri M, de Oliveira Piazetta MH, Gobbi AL, Kubota LT. 2010. Electrochemical detection in a paper-based separation device. *Anal. Chem.* 82(3):1162–65
35. Li C-Z, Vandenberg K, Prabhulkar S, Zhu X, Schneper L, et al. 2011. Paper based point-of-care testing disc for multiplex whole cell bacteria analysis. *Biosens. Bioelectron.* 26(11):4342–48
36. Li X, Tian J, Shen W. 2010. Quantitative biomarker assay with microfluidic paper-based analytical devices. *Anal. Bioanal. Chem.* 396(1):495–501
37. Lopez-Ruiz N, Curto VF, Erenas MM, Benito-Lopez F, Diamond D, et al. 2014. Smartphone-based simultaneous pH and nitrite colorimetric determination for paper microfluidic devices. *Anal. Chem.* 86(19):9554–62
38. Li X, Tian J, Shen W. 2010. Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors. *Cellulose* 17(3):649–59
39. Ratnarathorn N, Chailapakul O, Henry CS, Dungchai W. 2012. Simple silver nanoparticle colorimetric sensing for copper by paper-based devices. *Talanta* 99:552–57
40. Mentele MM, Cunningham J, Koehler K, Volckens J, Henry CS. 2012. Microfluidic paper-based analytical device for particulate metals. *Anal. Chem.* 84(10):4474–80
41. Cate DM, Nanthasurasak P, Riwkulkajorn P, L'Orange C, Henry CS, Volckens J. 2014. Rapid detection of transition metals in welding fumes using paper-based analytical devices. *Ann. Occup. Hyg.* 58(4):413–23

42. Dungchai W, Chailapakul O, Henry CS. 2010. Use of multiple colorimetric indicators for paper-based microfluidic devices. *Anal. Chim. Acta* 674(2):227–33
43. Sameenoi Y, Panymeesamer P, Supalakorn N, Koehler K, Chailapakul O, et al. 2012. Microfluidic paper-based analytical device for aerosol oxidative activity. *Environ. Sci. Technol.* 47(2):932–40
44. Karita S, Kaneta T. 2014. Acid-base titrations using microfluidic paper-based analytical devices. *Anal. Chem.* 86(24):12108–14
45. Liu H, Xiang Y, Lu Y, Crooks RM. 2012. Aptamer-based origami paper analytical device for electrochemical detection of adenosine. *Angew. Chem. Int. Ed.* 51(28):6925–28
46. Schonhorn JE, Fernandes SC, Rajaratnam A, Deraney RN, Rolland JP, Mace CR. 2014. A device architecture for three-dimensional, patterned paper immunoassays. *Lab Chip* 14(24):4653–58
47. Noh H, Phillips ST. 2010. Fluidic timers for time-dependent, point-of-care assays on paper. *Anal. Chem.* 82(19):8071–78
48. Govindarajan AV, Ramachandran S, Vigil GD, Yager P, Bohringer KF. 2012. A low cost point-of-care viscous sample preparation device for molecular diagnosis in the developing world; an example of microfluidic origami. *Lab Chip* 12(1):174–81
49. Jarujamrus P, Tian J, Li X, Siripinyanond A, Shiowatana J, Shen W. 2012. Mechanisms of red blood cells agglutination in antibody-treated paper. *Analyst* 137(9):2205–10
50. Renault C, Li X, Fosdick SE, Crooks RM. 2013. Hollow-channel paper analytical devices. *Anal. Chem.* 85(16):7976–79
51. Wang Y, Ge L, Wang P, Yan M, Ge S, et al. 2013. Photoelectrochemical lab-on-paper device equipped with a porous Au-paper electrode and fluidic delay-switch for sensitive detection of DNA hybridization. *Lab Chip* 13(19):3945–55
52. Noh H, Phillips ST. 2010. Metering the capillary-driven flow of fluids in paper-based microfluidic devices. *Anal. Chem.* 82(10):4181–87
53. Toley BJ, Wang JA, Gupta M, Buser JR, Lafleur LK, et al. 2015. A versatile valving toolkit for automating fluidic operations in paper microfluidic devices. *Lab Chip* 15(6):1432–44
54. Liu H, Li X, Crooks RM. 2013. Paper-based slippad for high-throughput chemical sensing. *Anal. Chem.* 85(9):4263–67
55. Connelly JT, Rolland JP, Whitesides GM. 2015. "Paper machine" for molecular diagnostics. *Anal. Chem.* 87(15):7595–7601
56. Liu H, Crooks RM. 2011. Three-dimensional paper microfluidic devices assembled using the principles of origami. *J. Am. Chem. Soc.* 133(44):17564–66
57. Martinez AW, Phillips ST, Whitesides GM. 2008. Three-dimensional microfluidic devices fabricated in layered paper and tape. *PNAS* 105(50):19606–11
58. Müller RH, Clegg DL. 1949. Automatic paper chromatography. *Anal. Chem.* 21(9):1123–25
59. Cuartero M, Crespo GA, Bakker E. 2015. Paper-based thin-layer coulometric sensor for halide determination. *Anal. Chem.* 87(3):1981–90
60. Wang P, Ge L, Yan M, Song X, Ge S, Yu J. 2012. Paper-based three-dimensional electrochemical immunodevice based on multi-walled carbon nanotubes functionalized paper for sensitive point-of-care testing. *Biosens. Bioelectron.* 32(1):238–43
61. Ge S, Ge L, Yan M, Song X, Yu J, Huang J. 2012. A disposable paper-based electrochemical sensor with an addressable electrode array for cancer screening. *Chem. Commun.* 48(75):9397–99
62. Lu J, Ge S, Ge L, Yan M, Yu J. 2012. Electrochemical DNA sensor based on three-dimensional folding paper device for specific and sensitive point-of-care testing. *Electrochim. Acta* 80:334–41
63. Tseng S-C, Yu C-C, Wan D, Chen H-L, Wang LA, et al. 2012. Eco-friendly plasmonic sensors: using the photothermal effect to prepare metal nanoparticle-containing test papers for highly sensitive colorimetric detection. *Anal. Chem.* 84(11):5140–45
64. Jokerst JC, Adkins JA, Bisha B, Mentele MM, Goodridge LD, Henry CS. 2012. Development of a paper-based analytical device for colorimetric detection of select foodborne pathogens. *Anal. Chem.* 84(6):2900–7
65. Pollock NR, Rolland JP, Kumar S, Beattie PD, Jain S, et al. 2012. A paper-based multiplexed transaminase test for low-cost, point-of-care liver function testing. *Sci. Transl. Med.* 4(152):152ra129

66. Xu M, Bunes BR, Zang L. 2011. Paper-based vapor detection of hydrogen peroxide: colorimetric sensing with tunable interface. *ACS Appl. Mater. Interfaces* 3(3):642–47
67. Eaidkong T, Mungkarndee R, Phollookin C, Tumcharern G, Sukwattanasinitt M, Wacharasindhu S. 2012. Polydiacetylene paper-based colorimetric sensor array for vapor phase detection and identification of volatile organic compounds. *J. Mater. Chem.* 22(13):5970–77
68. Maruo YY, Akaoka K, Nakamura J. 2010. Development and performance evaluation of ozone detection paper using azo dye orange I: effect of pH. *Sens. Actuators B* 143(2):487–93
69. Veigas B, Jacob JM, Costa MN, Santos DS, Viveiros M, et al. 2012. Gold on paper-paper platform for Au-nanoprobe TB detection. *Lab Chip* 12(22):4802–8
70. Bhakta SA, Borba R, Taba M Jr, Garcia CD, Carrilho E. 2014. Determination of nitrite in saliva using microfluidic paper-based analytical devices. *Anal. Chim. Acta* 809:117–22
71. Kim S, Jung E, Kim MJ, Pyo A, Palani T, et al. 2012. A simple, fast, and easy assay for transition metal-catalyzed coupling reactions using a paper-based colorimetric iodide sensor. *Chem. Commun.* 48(70):8751–53
72. Klasner S, Price A, Hoeman K, Wilson R, Bell K, Culbertson C. 2010. Paper-based microfluidic devices for analysis of clinically relevant analytes present in urine and saliva. *Anal. Bioanal. Chem.* 397(5):1821–29
73. Rattanarat P, Dungchai W, Cate DM, Siangproh W, Volckens J, et al. 2013. A microfluidic paper-based analytical device for rapid quantification of particulate chromium. *Anal. Chim. Acta* 800:50–55
74. Cate DM, Dungchai W, Cunningham JC, Volckens J, Henry CS. 2013. Simple, distance-based measurement for paper analytical devices. *Lab Chip* 13(12):2397–404
75. Apilux A, Siangproh W, Praphairaksit N, Chailapakul O. 2012. Simple and rapid colorimetric detection of Hg(II) by a paper-based device using silver nanoplates. *Talanta* 97:388–94
76. Das P, Ghosh A, Bhatt H, Das A. 2012. A highly selective and dual responsive test paper sensor of $Hg^{2+}/Cr^{3+}$ for naked eye detection in neutral water. *RSC Adv.* 2(9):3714–21
77. Hossain SMZ, Brennan JD. 2011. β-Galactosidase-based colorimetric paper sensor for determination of heavy metals. *Anal. Chem.* 83(22):8772–78
78. Zhang Y, Li X, Li H, Song M, Feng L, Guan Y. 2014. Postage stamp-sized array sensor for the sensitive screening test of heavy-metal ions. *Analyst* 139(19):4887–93
79. Chen G-H, Chen W-Y, Yen Y-C, Wang C-W, Chang H-T, Chen C-F. 2014. Detection of mercury(II) ions using colorimetric gold nanoparticles on paper-based analytical devices. *Anal. Chem.* 86(14):6843–49
80. Cate DM, Noblitt SD, Volckens J, Henry CS. 2015. Multiplexed paper analytical device for quantification of metals using distance-based detection. *Lab Chip* 15(13):2808–18
81. Evans E, Moreira Gabriel EF, Benavidez TE, Tomazelli Coltro WK, Garcia CD. 2014. Modification of microfluidic paper-based devices with silica nanoparticles. *Analyst* 139(21):5560–67
82. Cha R, Wang D, He Z, Ni Y. 2012. Development of cellulose paper testing strips for quick measurement of glucose using chromogen agent. *Carbohydr. Polym.* 88(4):1414–19
83. Salles MO, Meloni GN, de Araujo WR, Paixao TRLC. 2014. Explosive colorimetric discrimination using a smartphone, paper device and chemometrical approach. *Anal. Methods* 6(7):2047–52
84. Pourreza N, Golmohammadi H. 2015. Application of curcumin nanoparticles in a lab-on-paper device as a simple and green pH probe. *Talanta* 131:136–41
85. Wang W, Wu W-Y, Wang W, Zhu J-J. 2010. Tree-shaped paper strip for semiquantitative colorimetric detection of protein with self-calibration. *J. Chromatogr. A* 1217(24):3896–99
86. Zhou M, Yang M, Zhou F. 2014. Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe. *Biosens. Bioelectron.* 55:39–43
87. Wu X, Kuang H, Hao C, Xing C, Wang L, Xu C. 2012. Paper supported immunosensor for detection of antibiotics. *Biosens. Bioelectron.* 33(1):309–12
88. Yun S, Kim J. 2010. Multi-walled carbon nanotubes-cellulose paper for a chemical vapor sensor. *Sens. Actuators B* 150(1):308–13
89. Mirica KA, Weis JG, Schnorr JM, Esser B, Swager TM. 2012. Mechanical drawing of gas sensors on paper. *Angew. Chem. Int. Ed.* 51(43):10740–45
90. Apilux A, Dungchai W, Siangproh W, Praphairaksit N, Henry CS, Chailapakul O. 2010. Lab-on-paper with dual electrochemical/colorimetric detection for simultaneous determination of gold and iron. *Anal. Chem.* 82(5):1727–32

91. Lei KF, Lee K-F, Yang S-I. 2012. Fabrication of carbon nanotube-based pH sensor for paper-based microfluidics. *Microelectron. Eng.* 100:1–5
92. Jagadeesan KK, Kumar S, Sumana G. 2012. Application of conducting paper for selective detection of troponin. *Electrochem. Commun.* 20:71–74
93. Liu X, Mwangi M, Li X, O'Brien M, Whitesides GM. 2011. Paper-based piezoresistive MEMS sensors. *Lab Chip* 11(13):2189–96
94. Gimenez AJ, Yaánñez-Limoón JM, Seminario JM. 2011. Paper-based photoconductive infrared sensor. *J. Phys. Chem. C* 115(38):18829–34
95. Gimenez AJ, Yaánñez-Limoón JM, Seminario JM. 2010. ZnO-paper based photoconductive UV sensor. *J. Phys. Chem. C* 115(1):282–87
96. Gullapalli H, Vemuru VSM, Kumar A, Botello-Mendez A, Vajtai R, et al. 2010. Flexible piezoelectric ZnO-paper nanocomposite strain sensor. *Small* 6(15):1641–46
97. Mahadeva SK, Yun S, Kim J. 2011. Flexible humidity and temperature sensor based on cellulose-polypyrrole nanocomposite. *Sens. Actuators A* 165(2):194–99
98. Fu E, Liang T, Spicar-Mihalic P, Houghtaling J, Ramachandran S, Yager P. 2012. Two-dimensional paper network format that enables simple multistep assays for use in low-resource settings in the context of malaria antigen detection. *Anal. Chem.* 84(10):4574–79
99. Jin S-Q, Guo S-M, Zuo P, Ye B-C. 2015. A cost-effective Z-folding controlled liquid handling microfluidic paper analysis device for pathogen detection via ATP quantification. *Biosens. Bioelectron.* 63:379–83
100. Larsson PA, G Puttaswamaiah S, Ly C, Vanerek A, Hall JC, Drolet F. 2013. Filtration, adsorption and immunodetection of virus using polyelectrolyte multilayer-modified paper. *Colloids Surf. B* 101:205–9
101. Rattanarat P, Dungchai W, Cate D, Volckens J, Chailapakul O, Henry CS. 2014. Multilayer paper-based device for colorimetric and electrochemical quantification of metals. *Anal. Chem.* 86(7):3555–62
102. Zhang L, Wang Y, Ma C, Wang P, Yan M. 2015. Self-powered sensor for $Hg^{2+}$ detection based on hollow-channel paper analytical devices. *RSC Adv.* 5(31):24479–85
103. Jayawardane BM, McKelvie ID, Kolev SD. 2012. A paper-based device for measurement of reactive phosphate in water. *Talanta* 100:454–60
104. Sechi D, Greer B, Johnson J, Hashemi N. 2013. Three-dimensional paper-based microfluidic device for assays of protein and glucose in urine. *Anal. Chem.* 85(22):10733–37
105. Chen X, Chen J, Wang F, Xiang X, Luo M, et al. 2012. Determination of glucose and uric acid with bienzyme colorimetry on microfluidic paper-based analysis devices. *Biosens. Bioelectron.* 35(1):363–68
106. Mu X, Xin X, Fan C, Li X, Tian X, et al. 2015. A paper-based skin patch for the diagnostic screening of cystic fibrosis. *Chem. Commun.* 51(29):6365–68
107. Cunningham JC, Brenes NJ, Crooks RM. 2014. Paper electrochemical device for detection of DNA and thrombin by target-induced conformational switching. *Anal. Chem.* 86(12):6166–70
108. Nie Z, Nijhuis CA, Gong J, Chen X, Kumachev A, et al. 2010. Electrochemical sensing in paper-based microfluidic devices. *Lab Chip* 10(4):477–83
109. Santhiago M, Henry CS, Kubota LT. 2014. Low cost, simple three dimensional electrochemical paper-based analytical device for determination of p-nitrophenol. *Electrochim. Acta* 130:771–77
110. Dossi N, Toniolo R, Pizzariello A, Carrilho E, Piccin E, et al. 2012. An electrochemical gas sensor based on paper supported room temperature ionic liquids. *Lab Chip* 12(1):153–58
111. Novell M, Parrilla M, Crespo GA, Rius FX, Andrade FJ. 2012. Paper-based ion-selective potentiometric sensors. *Anal. Chem.* 84(11):4695–4702
112. Lan W-J, Zou XU, Hamedi MM, Hu J, Parolo C, et al. 2014. Paper-based potentiometric ion sensing. *Anal. Chem.* 86(19):9548–53
113. Zang D, Ge L, Yan M, Song X, Yu J. 2012. Electrochemical immunoassay on a 3D microfluidic paper-based device. *Chem. Commun.* 48(39):4683–85
114. Zhang L, Yang W, Yang Y, Liu H, Gu Z. 2015. Smartphone-based point-of-care testing of salivary α-amylase for personal psychological measurement. *Analyst* 140(21):7399–406
115. Arena A, Donato N, Saitta G, Bonavita A, Rizzo G, Neri G. 2010. Flexible ethanol sensors on glossy paper substrates operating at room temperature. *Sens. Actuators B* 145(1):488–94
116. Cheng C-M, Martinez AW, Gong J, Mace CR, Phillips ST, et al. 2010. Paper-based ELISA. *Angew. Chem. Int. Ed.* 49(28):4771–74

117. Määttänen A, Fors D, Wang S, Valtakari D, Ihalainen P, Peltonen J. 2011. Paper-based planar reaction arrays for printed diagnostics. *Sens. Actuators B* 160(1):1404–12
118. Funes-Huacca M, Wu A, Szepesvari E, Rajendran P, Kwan-Wong N, et al. 2012. Portable self-contained cultures for phage and bacteria made of paper and tape. *Lab Chip* 12(21):4269–78
119. Mensah ST, Gonzalez Y, Calvo-Marzal P, Chumbimuni-Torres KY. 2014. Nanomolar detection limits of $Cd^{2+}$, $Ag^{+}$, and $K^{+}$ using paper-strip ion-selective electrodes. *Anal. Chem.* 86(15):7269–73
120. Godino N, Gorkin R, Bourke K, Ducree J. 2012. Fabricating electrodes for amperometric detection in hybrid paper/polymer lab-on-a-chip devices. *Lab Chip* 12(18):3281–84
121. Dungchai W, Chailapakul O, Henry CS. 2009. Electrochemical detection for paper-based microfluidics. *Anal. Chem.* 81(14):5821–26
122. Santhiago M, Wydallis JB, Kubota LT, Henry CS. 2013. Construction and electrochemical characterization of microelectrodes for improved sensitivity in paper-based analytical devices. *Anal. Chem.* 85(10):5233–39
123. Rattanarat P, Dungchai W, Siangproh W, Chailapakul O, Henry CS. 2012. Sodium dodecyl sulfate-modified electrochemical paper-based analytical device for determination of dopamine levels in biological samples. *Anal. Chim. Acta* 744:1–7
124. Delaney JL, Hogan CF, Tian J, Shen W. 2011. Electrogenerated chemiluminescence detection in paper-based microfluidic sensors. *Anal. Chem.* 83(4):1300–1306
125. Tan SN, Ge L, Wang W. 2010. Paper disk on screen printed electrode for one-step sensing with an internal standard. *Anal. Chem.* 82(21):8844–47
126. Shi J, Tang F, Xing H, Zheng H, Lianhua B, Wei W. 2012. Electrochemical detection of Pb and Cd in paper-based microfluidic devices. *J. Braz. Chem. Soc.* 23:1124–30
127. Gribble CM, Matthews GP, Laudone GM, Turner A, Ridgway CJ, et al. 2011. Porometry, porosimetry, image analysis and void network modelling in the study of the pore-level properties of filters. *Chem. Eng. Sci.* 66(16):3701–9
128. Glavan AC, Martinez RV, Maxwell EJ, Subramaniam AB, Nunes RMD, et al. 2013. Rapid fabrication of pressure-driven open-channel microfluidic devices in omniphobic $R^F$ paper. *Lab Chip* 13(15):2922–30
129. Liu W, Chen D, Du W, Nichols KP, Ismagilov RF. 2010. Slipchip for immunoassays in nanoliter volumes. *Anal. Chem.* 82(8):3276–82
130. Lutz BR, Trinh P, Ball C, Fu E, Yager P. 2011. Two-dimensional paper networks: programmable fluidic disconnects for multi-step processes in shaped paper. *Lab Chip* 11(24):4274–78
131. Fu E, Kauffman P, Lutz B, Yager P. 2010. Chemical signal amplification in two-dimensional paper networks. *Sens. Actuators B* 149(1):325–28
132. Fu E, Lutz B, Kauffman P, Yager P. 2010. Controlled reagent transport in disposable 2D paper networks. *Lab Chip* 10(7):918–20
133. Toley BJ, McKenzie B, Liang T, Buser JR, Yager P, Fu E. 2013. Tunable-delay shunts for paper microfluidic devices. *Anal. Chem.* 85(23):11545–52
134. Lutz B, Liang T, Fu E, Ramachandran S, Kauffman P, Yager P. 2013. Dissolvable fluidic time delays for programming multi-step assays in instrument-free paper diagnostics. *Lab Chip* 13(14):2840–47
135. Chen H, Cogswell J, Anagnostopoulos C, Faghri M. 2012. A fluidic diode, valves, and a sequential-loading circuit fabricated on layered paper. *Lab Chip* 12(16):2909–13
136. Osborn JL, Lutz B, Fu E, Kauffman P, Stevens DY, Yager P. 2010. Microfluidics without pumps: reinventing the T-sensor and H-filter in paper networks. *Lab Chip* 10(20):2659–65
137. Rezk AR, Qi A, Friend JR, Li WH, Yeo LY. 2012. Uniform mixing in paper-based microfluidic systems using surface acoustic waves. *Lab Chip* 12(4):773–79
138. Chou J, Wong J, Christodoulides N, Floriano P, Sanchez X, McDevitt J. 2012. Porous bead-based diagnostic platforms: bridging the gaps in healthcare. *Sensors* 12(11):15467
139. Dungchai W, Sameenoi Y, Chailapakul O, Volckens J, Henry CS. 2013. Determination of aerosol oxidative activity using silver nanoparticle aggregation on paper-based analytical devices. *Analyst* 138(22):6766–73
140. Ornatska M, Sharpe E, Andreescu D, Andreescu S. 2011. Paper bioassay based on ceria nanoparticles as colorimetric probes. *Anal. Chem.* 83(11):4273–80

141. Luckham RE, Brennan JD. 2010. Bioactive paper dipstick sensors for acetylcholinesterase inhibitors based on sol-gel/enzyme/gold nanoparticle composites. *Analyst* 135(8):2028–35
142. Sun G, Liu H, Zhang Y, Yu J, Yan M, et al. 2015. Gold nanorods-paper electrode based enzyme-free electrochemical immunoassay for prostate specific antigen using porous zinc oxide spheres–silver nanoparticles nanocomposites as labels. *New J. Chem.* 39:6062–67
143. Li L, Kong Q, Zhang Y, Dong C, Ge S, Yu J. 2015. A 3D electrochemical immunodevice based on a porous Pt-paper electrode and metal ion functionalized flower-like Au nanoparticles. *J. Mater. Chem. B* 3(14):2764–69
144. Mostafalu P, Sonkusale S. 2015. A high-density nanowire electrode on paper for biomedical applications. *RSC Adv.* 5(12):8680–87
145. Savolainen A, Zhang Y, Rochefort D, Holopainen U, Erho T, et al. 2011. Printing of polymer microcapsules for enzyme immobilization on paper substrate. *Biomacromolecules* 12(6):2008–15
146. Guerrero MP, Bertrand F, Rochefort D. 2011. Activity, stability and inhibition of a bioactive paper prepared by large-scale coating of laccase microcapsules. *Chem. Eng. Sci.* 66(21):5313–20
147. Shiroma LY, Santhiago M, Gobbi AL, Kubota LT. 2012. Separation and electrochemical detection of paracetamol and 4-aminophenol in a paper-based microfluidic device. *Anal. Chim. Acta.* 725:44–50
148. Luo L, Li X, Crooks RM. 2014. Low-voltage origami-paper-based electrophoretic device for rapid protein separation. *Anal. Chem.* 86(24):12390–97
149. Ge L, Wang S, Ge S, Yu J, Yan M, et al. 2014. Electrophoretic separation in a microfluidic paper-based analytical device with an on-column wireless electrogenerated chemiluminescence detector. *Chem. Commun.* 50(43):5699–702
150. Tian J, Kannangara D, Li X, Shen W. 2010. Capillary driven low-cost V-groove microfluidic device with high sample transport efficiency. *Lab Chip* 10(17):2258–64
151. Gong MM, Zhang P, MacDonald BD, Sinton D. 2014. Nanoporous membranes enable concentration and transport in fully wet paper-based assays. *Anal. Chem.* 86(16):8090–97
152. Moghadam BY, Connelly KT, Posner JD. 2014. Isotachophoretic preconcentration on paper-based microfluidic devices. *Anal. Chem.* 86(12):5829–37
153. Moghadam BY, Connelly KT, Posner JD. 2015. Two orders of magnitude improvement in detection limit of lateral flow assays using isotachophoresis. *Anal. Chem.* 87(2):1009–17
154. Rosenfeld T, Bercovici M. 2014. 1000-fold sample focusing on paper-based microfluidic devices. *Lab Chip* 14(23):4465–74
155. Yuan J, Gaponik N, Eychmüller A. 2012. Application of polymer quantum dot-enzyme hybrids in the biosensor development and test paper fabrication. *Anal. Chem.* 84(11):5047–52
156. Ge L, Wang P, Ge S, Li N, Yu J, et al. 2013. Photoelectrochemical lab-on-paper device based on an integrated paper supercapacitor and internal light source. *Anal. Chem.* 85(8):3961–70