An Ultra-Fast and Large-Scale Fabrication Method for Paper-Based Microfluidic Chips

Hao Sun, Hui Dong and Jianping Zheng

Abstract Portable electronic devices and wireless communication enable a broad range of applications including environmental surveillance, food safety monitoring and point-of-care testing. Particularly, by incorporating smartphone and microfluidic paper-based device, rapid analysis, mobile laboratories for chemical assay, remote sensing and data management become more available. In this work, we designed and fabricated a 12-unit paper chip for colorimetric detection of metals. 48 chips can be obtained on a single A4 size paper within 5 min. Additive manufacturing technology was employed to construct the experimental platform. A cellphone's camera was used to capture colorimetric images, and then the RGB values of the color images were converted to grayscale via a self-built App. The intensity reflecting metal concentrations were processed and analyzed by the same app and a remote server. To demonstrate the utility of this approach, Fe solutions were tested. The detection limit of this method is 0.2 μg. The presented device and data processing tools hold a potential to assist mobile sensing, pollution detection and healthcare in routine work.

Keywords Paper-based microfluidics · Image processing by smartphone
Metal detection

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1 Introduction

Human exposure to metal-absorbing particulate matter has been studied broadly [1]. Epidemiological investigations of metal exposure in the workplace have found that such occupations as metalworking, construction, transportation, and mining place individuals at an increased risk to cardiovascular and respiratory health issues reducing the life expectancy of the population worldwide [2]. Tens of millions of individuals are exposed to metal-absorbing particulate matter in their workplace, but few are routinely monitored for their exposure due to the time-intensive sampling and unavailable cost analytical approaches [3]. Conventional tools for metals measuring include Atomic Absorption Spectroscopy (AAS) [4], Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [5], Mass Spectrometry (MS) [6]. Although these measurements are highly precise, sensitive with low detection limits, the testing costs are above $100 per sample in common and require trained specialists for operation which limits the application in developing countries where resources and experts are limited [3].

Paper sensors or μPADs are attractive as an analytical tool in which the sample flow is passively driven by capillary action with millimeter dimensions. μPADs have shown potential for overcoming technical and financial limitations in traditional methods [7, 8]. As a cheap, abundant, disposable, lightweight and biocompatible material, cellulosic paper is a natural platform for microfluidics [9]. In the past five years, numbers of fabrication methods for the patterning of paper substrates have been reported [10–14]. Theoretically, the objective is to create barriers on paper to prevent the aqueous samples from leaving the hydrophilic microfluidic structures. Initially, the fabrication was based on photolithography [15] followed by printed photomasks [16]. While the standard photolithography is able to obtain high structural resolution, the main limitation of this method is exposing the entire substrate to photoresist and organic solvents which are often toxic [17]. Moreover, the expensive photoresist is wasted inevitably, and redundant operation steps by different pieces of equipment are required (e.g. spinner, mask aligner, hot plates). Recently, alternative methods such as plasma etching [18], wax printing [19], laser cutting [20], flexographic printing [21] and wax screen-printing [22] have been developed. These methods have their advantages and disadvantages in terms of the number of operation, cost, speed, resolution, flexibility in pattern variation, and simplicity. However, one limitation all these methods share in common is the fabrication efficiency which is significant for testing throughput in downstream assays.

Application of μPADs is particularly adopted in point-of-need settings that require rapid analysis with low cost and simple operation. Over the past years, many paper sensors have been employed for clinical diagnostics [23], environmental monitoring [24], and food safety assurance [25]. Of these, by incorporating microfluidic paper-based device and human particulate matter exposure study, rapid simplified and effective quantitative analysis can be achieved. In particular, colorimetric detection is employed by most μPADs as it can provide easy readout and

clear profile of the generated chemical signals, making the instrument-free measurements available. The chromogenic reagents are chosen to selectively react with the analyte with minimal interference from other substances. To enable analyte quantification, detectors such as charge-coupled devices (CCD) and complementary metal-oxide sensors (CMOS) embedded in cameras or flatbed scanners have been widely employed. Then, image-processing software is used to quantify the color signal intensities and/or hues reflecting the chemicals concentrations.

In this work, we incorporated smartphone and microfluidic paper-based device to set up a mobile laboratory for rapid and simplified metal sensing. We designed and fabricated a 12-unit paper chip for colorimetric detection of Fe. Cellphone's camera was used to capture colorimetric images, and then the RGB values of the color images were converted to grayscale via a custom designed app. The intensity reflecting metal concentrations were processed and analyzed by the same app and a remote server. Log-linear calibration curves were generated for each metal, with method detection limits ranging from 0.2 to 2.0 μg. The presented device and data processing tools hold a potential to assist mobile sensing, pollution detection and healthcare in low-resource areas.

2 Materials and Chips

2.1 Materials

All reagents and materials were purchased from commercial suppliers and used as received. Octadecyl acrylate (Product #: A1011), 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651, Product #: D1702), 1,10-decanediol diacrylate (Product #: B2937), were purchased from Tokyo Chemical Industry (Shanghai warehouse, China). Whatman® qualitative filter paper, Grade 1 (Product #: 1001-917, 460 × 570 mm), Grade 3 (Product #: 1003-917, 460 × 570 mm), Grade 4 (Product #: 1004-917, 460 × 570 mm), were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Iron chloride hexahydrate (Cat. #: F102739), 1,10-Phenanthroline (Cat. #: P104932) and poly(acrylic acid) (Cat. #: P131659) were purchased from Aladdin Bio-Chem Technology (Shanghai, China). Glacial acetic acid (Cat. #: 2004012-01-01), sodium acetate anhydrous (Cat. #: 1001051-01-09) was purchased from Xilong Scientific (Shanghai, China). Laboratory containers were rinsed with deionization water prior to use.

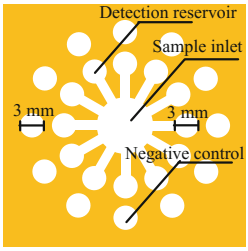
2.2 Chip Design

The μPAD design and dimensions are described in Fig. 1. A design was created using drawing software (CorelDRAW) in which twelve detection units and control

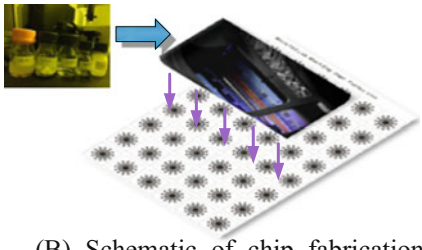
circles were patterned. In each unit, one pair of detection reservoir and channel was designed. The diameter of detection reservoir and control circle was 3.0 mm. The size of a single chip was 30.0 × 30.0 mm (orange part in Fig. 1a). On a single A4 size paper (210.0 × 297.0 mm), 48 chips were patterned as shown in Fig. 1b. The fabricated chips are shown in Fig. 1c.

2.3 *Fabrication*

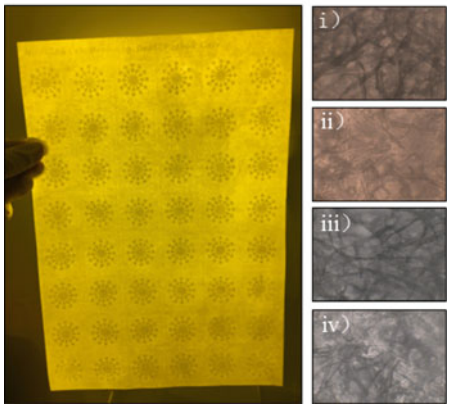
The chip fabrication was based on ink printing. In details, we modified an inkjet printer (Epson L800, Seiko Epson, Suwa, Japan) featuring a minimum ink droplet volume of 3 pL. The composition of the UV curable ink is given in Table 1. Whatman® qualitative filter papers (460 × 570 mm) were cut into pieces of A4 size, and then the paper was fixed onto A4 copy paper sheets and fed into the printer through the standard paper feeding mechanism. UV curing was performed by irradiation at a wavelength of 365 nm from two printer embedded UV diodes. The



(A) Chip design



(B) Schematic of chip fabrication (zone in black color is hydrophilic)



(C) Fabricated 48 chips on a single A4 size paper

Fig. 1 Paper-based microfluidic chip design and fabrication

Table 1 Constituent of the UV curable ink

Reagent	Function	Weight%
Octadecyl acrylate	Monomer	70.0
1,10-Decanediol diacrylate	Crosslinker	15.0
2,2-dimethoxy-2-phenylacetophenone	Photo initiator	15.0

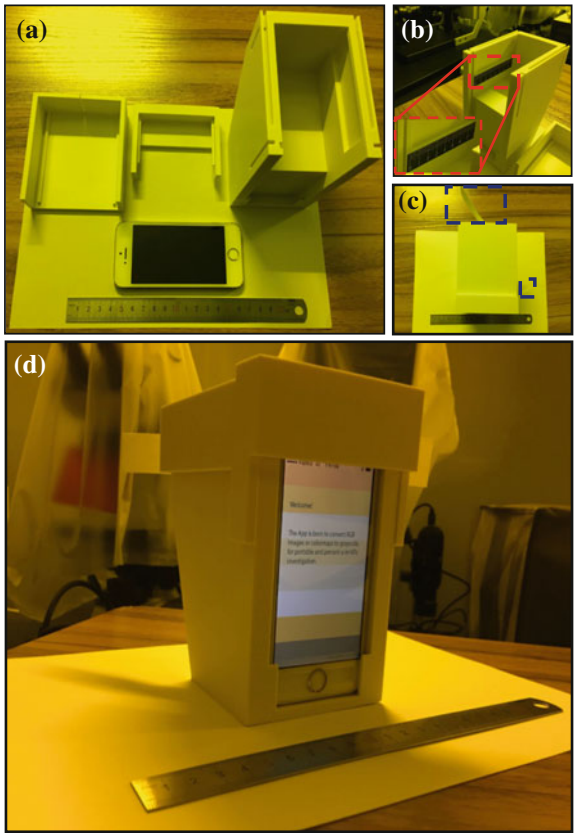


Fig. 2 The experimental set-up. **a** Modules of the shell fabricated by 3-D printing and a cellphone (Apple 5s). **b** Details of the bin's inside. **c** Top view of the assembled hardware. **d** The cellphone embedded experimental set-up (The welcome page is shown on cellphone screen)

printed paper was placed on a hot plate at 95 °C for 120 s or suspended overnight to consolidate the printed feature, forming the three-dimensional hydrophobic barriers that control liquid flow. The schematic of the fabrication process is shown in Fig. 2b. One side of the device was covered with clear packing tape to prevent solution from leaking out underneath the paper during the assay.

3 Experimental Set-up

The experimental set-up included two parts, namely the hardware and the app analytics. The hardware was used for fixing paper chips and refrain the difference in camera focal distance among each tests. The software was based on Mac OS and coded in Xcode via Swift language (version 3.0).

3.1 Hardware

Additive manufacturing or three-dimensional (3-D) printing technology was employed to construct the hardware. A desktop level 3-D printer (Finder®, Shanzhu Inc., Zhejiang, China) was used to create the 3-D object. Polylactide (PLA) material (a kind of biodegradable and bioactive thermoplastic aliphatic polyester) was heated to 210 °C at the nozzle zone and form layers of the structure. A 3-D printed hardware is shown in Fig. 2. Four components were included in the hardware platform, namely cover (with two inner slide rails, Fig. 2a top-left), front board (with two slide rails, Fig. 2a top-middle), bin (with four outer grooves and two inner cavities, Fig. 2a top-right) and cellphone. The two inner cavities were used for fixing paper chips precisely as shown in Fig. 2b. The total printing time cost was about 20.0 h using at fine resolution. Colorimetric images were captured by the cellphone's camera.

3.2 Image Processing App

Since the App's user interface can be constructed and managed by macOS easily, we created a software in the developer toolset Xcode (8.0) in Swift language (3.0). The capacity of the App included image capture (both in landscape and portrait directions) and processing which converted RGB information from image's pixels to grayscale. The image processing efficiency depended on the codes and CPU capacity of the phone. Generally, an image with 1136 × 640 pixels can be processed within 80 s. The schematic of image processing is shown in Fig. 3.

Fig. 3 Procedure of image processing process

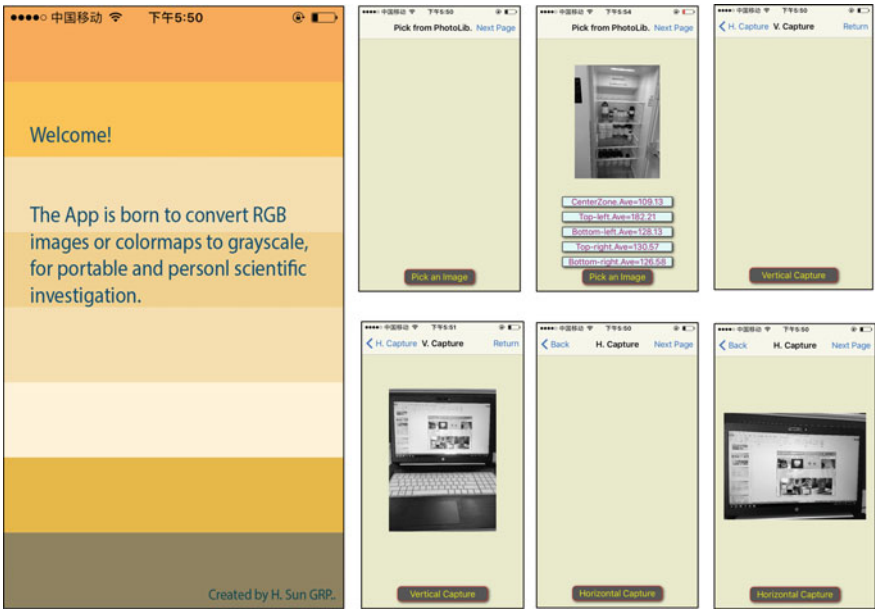


Fig. 4 The image processing software developed by the Apple Developer Program

Pages of the App's user interface are shown in Fig. 4.

4 Results and Discussion

4.1 Hydrophobicity Tests

At the room temperature and under standard atmospheric pressure, we tested the contact angles of solid surface of the fabricated paper chip hydrophobic zone. A contact angle meter (Precise Test Inc., Dongguan, China) was used to measure the angles after water droplet introduction by a syringe pump (Holliston, Massachusetts, United States). 3.0 μL DI water droplets were dropped on the solid surface and the contact angles were measured by the software provided by the manufacturer. We captured the angle variation at different time (0, 0.5 and 1.0 h) after droplet introduction as shown in Fig. 5.

The testing results based on Whatman® filter paper grade 4 substrate were shown in Fig. 5a–c, and the results from grade 1 paper were presented in Fig. 5d–f. The initial angles on each paper substrates (114.7° for grade 4 and 123.2° for grade 1) were beyond 90° indicating the surfaces were all hydrophobic. Also, the wettability of grade 4 paper by water was more obvious than grade 1. While, with time extension, the contact angle on grade 4 paper surface decreased by ~12.6° within

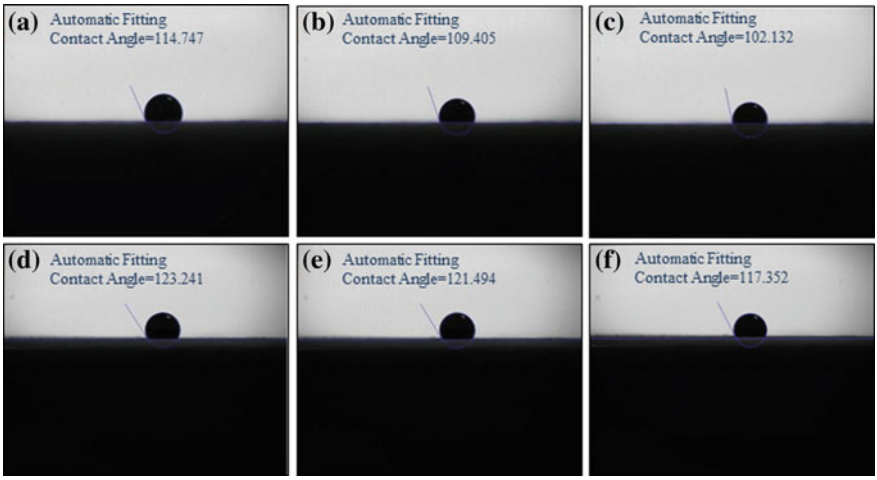


Fig. 5 Contact angles of water droplet on printed paper surfaces

1 h which was larger than grade 1 (~5.9°). The phenomenon was mainly caused by the difference in particle retention of different papers. Particle retention is an attribute of a papermaking process indicating that filler and/or other fine particles are retained in the finished paper. In printing process, the ink penetrated through the cellulose and cured by UV light irradiation. Grade 1 paper has a smaller particle retention size than grade 4, and therefore can absorb more liquid in an identical time. Also, the uniformity of the cured molecules on top surface of the grade 1 paper was better than grade 4 paper's.

4.2 Colorimetric Assays

The diffusion uniformity was first verified by pipetting hydrophilic dyes to the sample inlet zone. The Fig. 6a (top) demonstrates good profiles of on-chip dye diffusion. The ratio of detection reservoir area to total hydrophilic area can be calculated to be about 1:17.9. Thus, in the following tests, we introduced 17.9 volume unit Fe solution to the sample inlet zone to get 1 volume unit reach the detection reservoir.

An acetate buffer for the Fe assay was prepared with 5.0 g of sodium acetate trihydrate and 3.9 mL of glacial acetic acid in 50 mL of water. 1,10-phenanthroline was then added to the acetate buffer to obtain a concentration of 8 mg/mL. The μPAD was prepared for detection of Fe by first adding 0.5 μL of poly(acrylic acid) (0.7 mg/mL) to the detection reservoir, followed by two 1.0 μL aliquots of 1,10-phenanthroline also in the detection reservoir. The device was allowed to dry completely between each addition of reagent. Solid iron chloride hexahydrate was

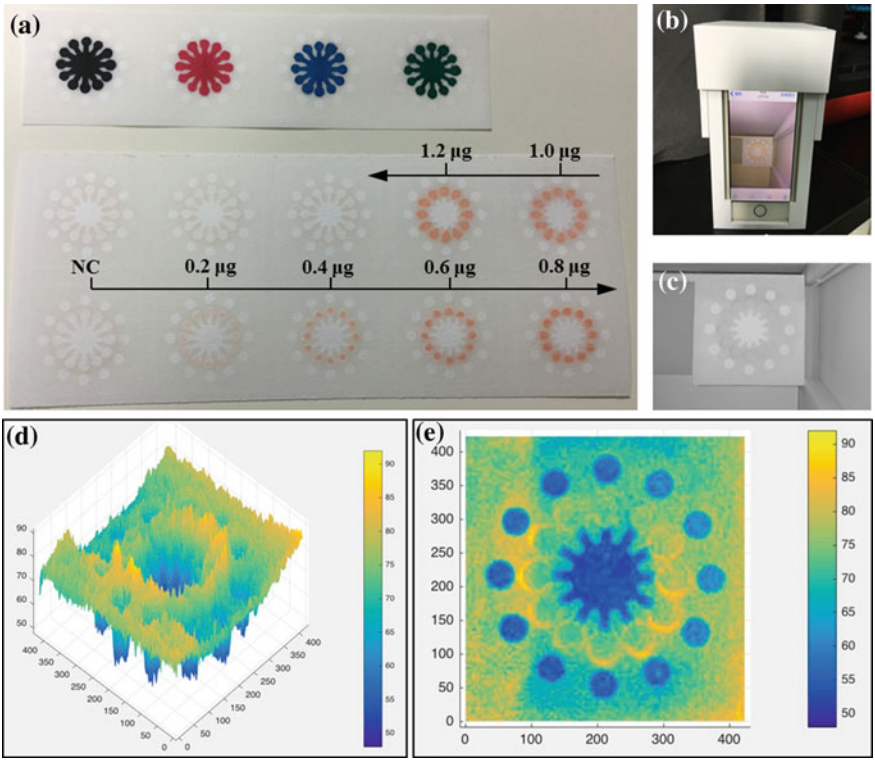


Fig. 6 Colorimetric Assays of low abundance Fe. **a** Hydrophilic dye diffusion tests (top) and colorimetric assays of Fe ions with amount varying from 0.2 to 2.0 μg (bottom). **b** Colorimetric images captured by the self-built App in an iphone. **c** The 3-D printed frame in white color makes the focal distance consistent. **d** Gray values of each pixel of the chip image plotted in Matlab (Parula color map). **e** Top view of (**d**)

added to DI water to get concentrations of 0.2–1.2 μg/μL with a step of 0.2 μg/μL. Then, Fe solution (17.9 μL) was pipetted to sample inlet zone of the paper chip and diffused to 12 detection reservoirs. After the detection reservoirs drying completely, we obtained the colorimetric images as presented in Fig. 6a (bottom).

The image capture process in self-developed App is shown in Fig. 6b. Also, in the identical App, the RGB image was converted to grayscale as presented in Fig. 6c. Then, the gray values of each pixel's were collected by a Macbook and plotted in the software Matlab (R2016A for mac). The digital figure results from the gray image of 0.2 μg/μL Fe solution are illustrated in Fig. 6d, e.

From Fig. 6d, e, the average gray values of 12 detections was about 82.1 and the average values of 12 negative circles was about 54.5. Thus, the proposed approach and paper based microfluidic chip is able to differentiate metal at very low abundance.

5 Conclusions

On a single A4 size filter paper, we designed and fabricated 48 paper chips for colorimetric detection of metals. The readout of each chip is 12. The total fabrication time cost is 5 min. Additive manufacturing technology was employed to construct the experimental hardware. By employing a usual cellphone's camera, the colorimetric images were captured. Through a self-built App the RGB values of the color images were converted to grayscale. The intensity reflecting metal concentrations were processed and analyzed by the same app and a remote server. Fe solutions were tested on-chip to demonstrate the utility of this approach. The detection limit of this method is 0.2 μg. The presented device and data processing tools hold a potential to simplify mobile sensing, pollution detection and healthcare in routine work.

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