

# Paper-based three-dimensional microfluidic device for monitoring of heavy metals with a camera cell phone

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Received: 11 December 2013 / Revised: 8 February 2014 / Accepted: 20 February 2014 / Published online: 12 March 2014  
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**Abstract** A 3D paper-based microfluidic device has been developed for colorimetric determination of selected heavy metals in water samples by stacking layers of wax patterned paper and double-sided adhesive tape. It has the capability of wicking fluids and distributing microliter volumes of samples from single inlet into arrays of detection zones without external pumps, thus a range of metal assays can be simply and inexpensively performed. We demonstrate a prototype of four sample inlets for up to four heavy metal assays each, with detection limits as follows: Cu (II)=0.29 ppm, Ni(II)=0.33 ppm, Cd (II)=0.19 ppm, and Cr (VI)=0.35 ppm, which provided quantitative data that were in agreement with values gained from atomic absorption. It has the ability to identify these four metals in mixtures and is immune to interferences from either nontoxic metal ions such as Na(I) and K(I) or components found in reservoir or beach water. With the incorporation of a portable detector, a camera mobile phone, this 3D paper-based microfluidic device should be useful as a simple, rapid, and on-site screening approach of heavy metals in aquatic environments.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00216-014-7715-x) contains supplementary material, which is available to authorized users.

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**Keywords** Microfluidics · Paper-based · Three-dimensional · Metal assay · Camera phone

## Introduction

Aquatic environments, including freshwater, estuaries, and coastal marine waters, are often contaminated with numerous organic and inorganic compounds. Among these pollutants, heavy metals cannot be easily detoxified via degradation, resulting in their persistence, and making them become some of the most frequent inorganic pollutants in these ecosystems. Many of these heavy metals (e.g., Cu (II), Cd (II), Pb (II), Hg (II), Cr (VI), and Ni (II)) can act as ecological toxins in aquatic ecosystems and may pose a health risk to humans and the environment [1–4]. Thus, monitoring of heavy metals in fresh and beach waters is very important.

Several pieces of equipment have been developed for the identification and quantification of these heavy metals, including inductively coupled plasma/atomic emission spectrometer (ICP/AES), inductively coupled plasma mass spectrometer (ICPMS), as well as atomic absorption spectrometer (AAS) [5–8]. Though many of these spectrometers are ultrasensitive and relatively accurate, they are expensive and generally not amenable to on-site testing by unskilled personnel. Thus, they could benefit from a more convenient device, which is inexpensive, rugged, lightweight, and independent of supporting infrastructure. Among the most successful of current systems are those that rely on lateral movement of fluids across paper strips to distribute reagents and bring about chromogenic results (e.g., optical test dipsticks and lateral flow microfluidic system) [9–14]. These systems are useful, but limited in their capabilities [15, 16]. Hence, we firstly introduce a well-established paper-based 3D flow design [17, 18] into

colorimetric determination of heavy metal ions together with a detector (a cell phone embedded with a camera) to make up a 3D paper-based microfluidic multiplex metal assay device. The paper-based 3D system is manufactured by layering paper patterned into hydrophilic channels and hydrophobic walls as well as tape patterned with holes that connect these channels in different layers of paper. This device expands paper-based metal assays from simple 1D lateral-flow system to 3D device with complex microfluidic paths, and expands significantly the capabilities of very low-cost system. Though there are many examples of 3D glass or polymeric microfluidic devices for metal assays [19–23], 3D paper-based microfluidic device combines simplicity in fabrication, complexity in fluidic and analytical capability, and low cost. While 3D paper-based microfluidic device use the capillary wicking to control flows, it requires no external equipment or power sources and thus without a doubt a promising technique for on-site detection of heavy metal ions.

A prototype of four sample inlets for up to four heavy metal assays each was developed in our experiments which is small ( $<15\text{ cm}^2$ ), lightweight ( $0.15\text{ g cm}^{-2}$ ), and low cost (approximately \$3). The notable modification in the present 3D paper-based prototype was that functional reagents (e.g., basification regulator, masking reagents, and chromogenic reagents) were separately preloaded into the different layers within the 3D paper system. Driven by capillary force, four sample streams were both vertically and laterally distributed and crossed another layer without mixing [24, 25]. While crossing, the sample streams were respectively combined with different type of reagents, or filtered, and finally into the complex arrays of detection zones within minutes. So it required relatively simple kits in comparison with those in lateral flow systems [9–14], and multiplex and high-density ( $4\times 4\times 4$ ) metal identifications can be simply performed. We use a cell phone with a camera as a detector to monitor the metal assay results on the paper-based microfluidics. The images can be analyzed on-site by a personal computer or transmitted by e-mail or MMS messaging combined in the cell phone to a central lab for further quantitative analysis [26, 27]. This detection method is more accurate than visual colorimetry; the usual way to measure in 1D later-flow systems and also has advantages of easy to use, low cost, portability, and mobility. The developed 3D paper-based microfluidic device which gathered the technologies of 3D paper-based system, colorimetric determination, and use of a smart phone camera as detector represents a first step in realizing such integration towards low-cost, ease of use, and portable multiplex heavy metal detection. It has a significant promise for rapid and low-cost environmental monitoring, especially in resources-limited regions.

## Materials and methods

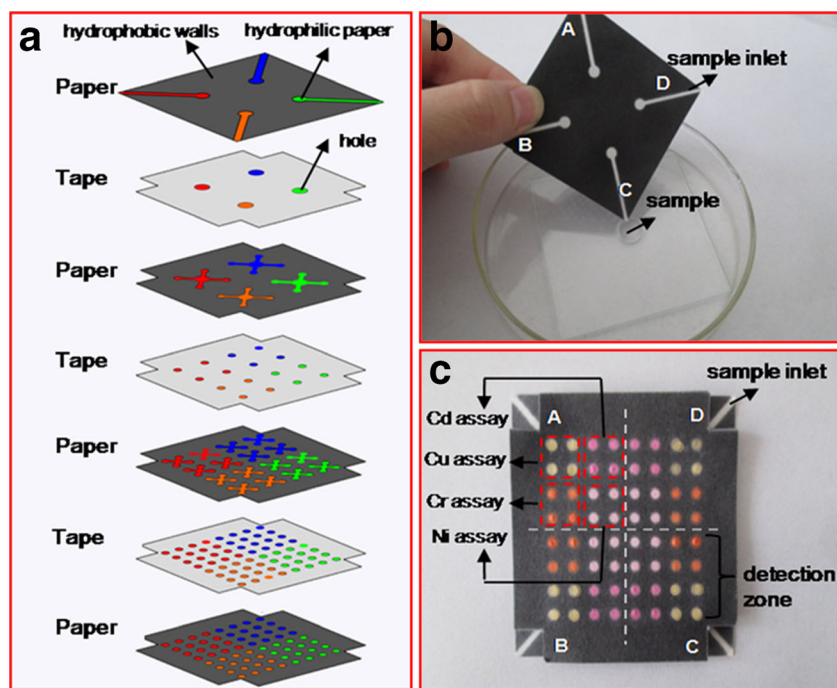
### Chemicals and solutions

NaCl, KCl, ammonium, acetone, Triton X-100, NaSCN, HF,  $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ ,  $\text{NiSO}_4\cdot 6\text{H}_2\text{O}$ ,  $\text{CdCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{Na}_2\text{S}_2\text{O}_3$ , sodium diethyldithiocarbamate, dimethylglyoxime, cadion, diphenylcarbazide, and ethylenediaminetetraacetic acid (EDTA) were purchased from J&K. Distilled deionized water ( $\text{ddH}_2\text{O}$ ) was obtained from Milli-Q Synthesis A10 water purification system. All other reagents were of analytical grade.

Heavy metal stock solutions were prepared using  $\text{ddH}_2\text{O}$  (pH 6) over the concentration range of 0.1–20 ppm. Sodium diethyldithiocarbamate was dissolved at a concentration of  $1.0\text{ mg ml}^{-1}$  in  $0.05\text{ mol l}^{-1}$  EDTA and  $0.05\text{ mol l}^{-1}$  HF, and the pH was adjusted to 8.5 using 2 % (v/v) ammonium hydroxide. Dimethylglyoxime was dissolved at  $2\text{ mg ml}^{-1}$  into 50 % (v/v) acetone with ammonium hydroxide and added  $0.05\text{ mol l}^{-1}$  EDTA and  $0.05\text{ mol l}^{-1}$   $\text{Na}_2\text{S}_2\text{O}_3$ . Cadion was dissolved at  $0.3\text{ mg ml}^{-1}$  into 50 % (v/v) acetone and added  $0.05\text{ mol l}^{-1}$  EDTA and  $0.05\text{ mol l}^{-1}$  HF. Diphenylcarbazide was dissolved at  $0.4\text{ mg ml}^{-1}$  into 50 % (v/v) acetone. And the 2 % (v/v) ammonium hydroxide, the 0.5 % (v/v)  $\text{H}_2\text{SO}_4$ , and the 0.5 % (v/v)  $\text{H}_3\text{PO}_4$  were also prepared.

### 3D paper microfluidics design and fabrication

The 3D paper-based microfluidic device in our experiment mainly integrated a microfabricated paper chip with a detector (a cell phone embedded with a camera). The layout of the paper chip was shown in Fig. 1a, which was composed of four paper (Whatman No.1, USA) layers and three tape layers. For the paper layers, the hydrophobic walls (denoted as black area) were printed using a Xerox Phaser 8560 wax printer and the blank regions (denoted as colorized zone) were maintained hydrophilic paper to drive flows both vertically and laterally. The tape layers were patterned by holes (filled with cellulose powder and reagents) that connected channels in different layers of paper. The top paper layer had four sample inlets (denoted as A, B, C, and D), which enable four assays simultaneously (Fig. 1b). The sample streams were distributed and cross another layer that were combined with different reagents in different layers, or filtered (as one of many possible functions) and arrived at the array of detection zones ultimately of the bottom layer. The array of detection zones was separated in to four regions (denoted as A, B, C, and D) corresponding to four samples, and  $4\times 4$  metal identifications were allowed in each region. The chromogenic results can be visible and detected from the circular regions (with a diameter of 3.0 mm) of the detection array (Fig. 1c). The total paper chip size was 5.5-cm long, 5.5-cm wide, and 2-mm thick. The fabrication process of the paper chip mainly includes



**Fig. 1** 3D paper-based microfluidic chip design and fabrication. **a** Layout of the chip that is composed of four paper layers and three tape layers. For the paper layers, the hydrophobic walls (denoted as *black area*) were printed using a Xerox Phaser 8560 wax printer and the blank regions (denoted as *colorized zone*) were maintained hydrophilic paper to drive flows both vertically and laterally. The tape layers were patterned by holes that connected channels in different layers of paper. **b** Photograph of the top of the 3D paper-based chip which has four sample inlets (denoted as

*A, B, C, and D*) and enable four assays simultaneously. **c** Photograph of bottom of the 3D paper-based chip dipped into metal contaminated water. Each of the detection zones undergoes the corresponding color change (*brown color, pink-red color, yellow-red color, and red magenta color* in the presence of Cu (II), Ni(II), Cd(II), and Cr (VI), respectively). The array of detection zones is separated four regions (denoted as *A, B, C, and D*) corresponding four samples and  $4 \times 4$  metal identifications are allowed in each region

patterning the paper and attaching and assembling the chip (see Electronic Supplementary Material Fig. S1). In the step of patterning paper, we used filter paper (Whatman No.1, USA) as the paper layer and ACE double-sided carpet tape, although other types of paper and tape can be used as well. The patterns were designed using the mapping software (Freehand, 11.0.1, Adobe) and printed onto the surface of the filter paper with a Xerox Phaser 8560 wax printer and the tape with a HP Deskjet 2520hc printer. The wax-printed filter papers were baked in an oven at 120 °C for 5 min to let the printed wax melt and penetrate through the paper to form the hydrophobic patterns. It would be ready for use after cooling to room temperature. The water-impermeable double-sided tapes were holed according to the printed patterns by a punch. Those procedures could be finished within 10 min.

After manufacturing every layer of paper and tape, we arranged them in the correct sequence. One face of the correctly-oriented double-sided tape was attached to the bottom layer of the paper. The other face of the double-sided tape remained protected by the plastic backing supplied with the tape. The holes in the tape were filled with a mash made of cellulose powder, reagents, and water and then dried in the vacuum airing apparatus (Boxun, DZF 6050, Shanghai, China) at 25 °C for 5 min. After that, the plastic baking was peeled from the tape leaving the mash paste in the holes. Repeating

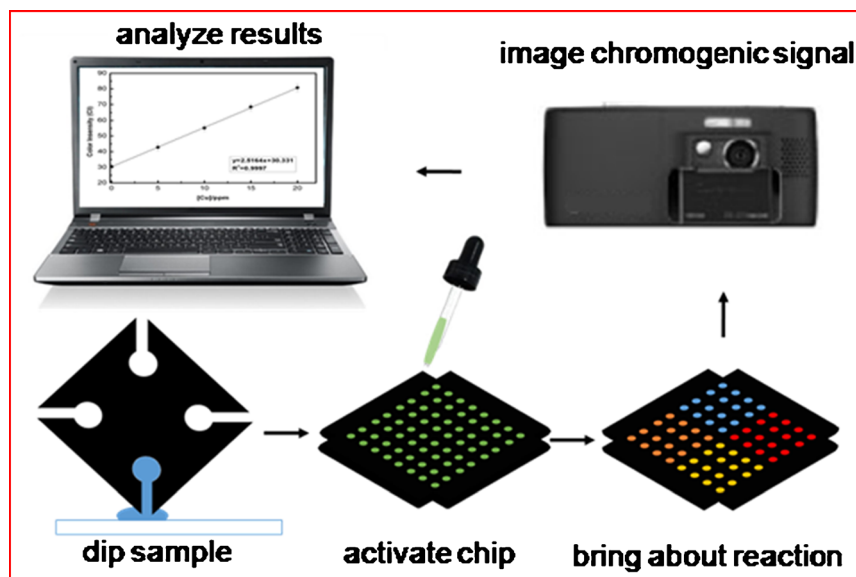
the stacking procedure until the top layer of the paper was assembled, and the entire chip was compressed by rolling three times with a plastic rolling pin. After assembling, the paper chip was sealed with plastic films using a heat seal machine and is ready for use.

The reagents within the paper chip, mainly including masking reagents and metal selective chromogenic reagents, were filled into the corresponding holes of the middle tape layer and the bottom tape layer, respectively. The masking reagents were sodium malonate (20 %) and NaF (15 %) for detection of Cu (II) and  $\text{Na}_2\text{S}_2\text{O}_3$  (40 ppm), 0.05 % (*v/v*)  $\text{H}_2\text{SO}_4$  for detection of Ni (II) and thiourea (40 ppm), NaF (15 %) for detection of Cd (II), and NaSCN (40 ppm) for detection of Cr (VI). The metal-selective chromogenic included sodium diethyldithiocarbamate for detection of Cu (II), dimethylglyoxime for detection of Ni (II), cation for detection of Cd (II), and diphenylcarbazide for detection of Cr (VI).

### 3D paper microfluidic device operation

After the paper chip was assembled, there were three steps to complete the metal assays on the paper chip (Fig. 2). First, four samples (30  $\mu\text{l}$  each) were dipped in turn for 1 min into the paper chip from the four sample inlets. Driven by capillary force, four sample streams were distributed and crossed

**Fig. 2** Schematic illustration of metal assay based on 3D paper microfluidics. Samples are dipped into the paper chip from sample inlets. After sample streams reaching into detection zones, activation solutions are dropped to bring about metal chromogenic reactions. The chromogenic signals are imaged by a camera cell phone and analyzed in a personal computer with image processing and analysis software



another layer and finally into the complex arrays of detection zones. While crossing the tape layers, the sample streams were combined in turn with different type of reagents including masking reagents and metal-selective chromogenic reagents. And then, activation solutions (1  $\mu\text{l}$  each) were dropped separately into the matched detection zones to bring about metal chromogenic reactions. Only three activation solutions, organic solution (acetone) for Ni (II) assay, surfactant (TritonX-100) for Cd (II), and acidification regulator (0.5 % (v/v)  $\text{H}_2\text{SO}_4$ , 0.5 % (v/v)  $\text{H}_3\text{PO}_4$ ) for Cr (VI), were used here. After that, we used a cell phone embedded with a camera as detector to monitor the metal identification results on-chip. To bring light conditions into consistency, the paper-based chip was placed into an opaque with an inner LED. The chromogenic signal of the detection zones on-chip was imaged by a camera cell phone (13 megapixels,  $\times 4$  digital zoom). The images can be analyzed in a personal computer with image processing and analysis software by converting each image to an eight-bit grayscale mode and measuring the average grayscale values within the circular regions. Image-Pro Plus software (version 6.0 for Windows XP; Media Cybernetics) was used in our experiment.

#### Sample distribution and reagent validity in 3D paper microfluidics

To examine sample distribution in 3D paper chip, eight increasing concentrations of aqueous dye (Amaranth) (30  $\mu\text{l}$  each) were dipped into the paper chip from the four sample inlets. Two separate paper chips were used here. The intensity of different concentrations of dye in the detection zones were tested as outlined above. To conduct a stability study on 3D paper microfluidics at 25  $^\circ\text{C}$ , four heavy metal ions including Cu (II), Ni (II), Cd (II), and Cr (VI) at known concentrations

were assayed using 3D paper microfluidics first at initial time and then at 2, 4, and 6 months time points, respectively.

#### Paper microfluidics validation and heavy metal assays

Four heavy metal ions including Cu (II), Ni (II), Cd (II), and Cr (VI) dissolved in ddH<sub>2</sub>O at known different concentrations (30  $\mu\text{l}$  each) were dipped into the paper chip, respectively. The color intensities of the detection zones were monitored as outlined above. The detection zone incubated with activation solutions and absent of heavy metals was set as the control. The chromogenic results were analyzed and the standard curves were constructed by plotting the color intensity values against metal concentrations using Origin Pro 8.0. All data points represented the average values obtained from the parallel four detection zones, and the error bars represented the standard deviations (SD) of those points, with detection limits representing signals above 3 SD of the control signal. For validation study of 3D paper microfluidics, four heavy metal ions including Cu (II), Ni (II), Cd (II), and Cr (VI) at known high and low concentrations were assayed first using 3D paper microfluidics and then by atomic absorption spectroscopy (AAAnalyst 400 AA spectrometer).

#### Selectivity and interference study

To examine the ability to quantify heavy metal selectively, samples (30  $\mu\text{l}$  each) containing individual metal ion separately (Cu (II) (10 ppm), Ni (II) (10 ppm), Cd(II) (0.2 ppm), or Cr (VI), (0.5 ppm)) and a mixture of metals (Cu (II) (10 ppm), Ni (II) (10 ppm), Cd(II) (0.2 ppm), or Cr (VI), (0.5 ppm)) were dipped into the paper chip. Two separate paper chips were used here, with two individual metal assays, one mixture assay, and one control in each chip. The color intensity was

monitored as described above. To examine interferences by concomitant ions, a fixed amount of NaCl (200 ppm) or KCl (100 ppm) was mixed with different concentrations of heavy metal ions. The chip was dipped with sample for 1 min and tested as outlined above. Interferences were also examined by spiking reservoir water and beach water with a fixed amount of heavy metal ions and testing as outlined above.

## Result and discussion

### 3D paper microfluidic device characterization

In this study, we develop an analytical device fabricated by stacking alternating layers of paper and water-impermeable double-sided adhesive tape. The hydrophobic wax patterned into the paper delimited channels through which the fluids moved laterally; the water-impermeable tape separated channels in neighboring layers of paper, and holes punched into the tape enable vertical flow. Thus, the device extended paper-based chip from 1D lateral-flow system to 3D system with complex microfluidic paths. A prototype of four sample inlets for up four heavy metal assays each was demonstrated and its capability of moving fluids in 3D was illustrated in Fig. 3a. Samples absorbed from sample inlets on the top layer of the device can be distributed into an array of detection zones on the bottom layer of the device. The prototype showed in Fig. 3b distributed each of the eight different concentrations of amaranth (red dye) into arrays of detection zones. The intensity of red in the inlet spot were compared with the average values in each region of 16 zones. No significant differences ( $p > 0.05$ ) between the intensities at the inlet spots and the detection zones, and the small standard deviations of the measurements indicated that the device evenly distributed the samples without loss due to adsorption (Fig. 3c). The ability to distribute samples into multiple detection zones using 3D paper microfluidics made it possible to measure simultaneously the concentrations of solutes (using colorimetric assays) in a sample and to generate calibration curves for the assays. In the present prototype, four analyses simultaneously detecting in each of the four metals and four replicates for each metal ( $4 \times 4 \times 4$ ) can be performed. Flexibility in design and easy patterning by wax printing can provide an alternative detection array. A high-density patterning would make it possible to simultaneously conduct high-throughput screening.

For metal detection, the most simplified activation solutions were used, thus it did not need external complex kit like 1D later-flow systems [9–14]. Only three varieties of activation solutions were needed. They were organic dissolvent (acetone) for the dissolution of fat-soluble chromogenic reagents in the Ni (II) detection, surfactant (TritonX-100) for Cd (II) complex, and acidification solution for pH adjustment of

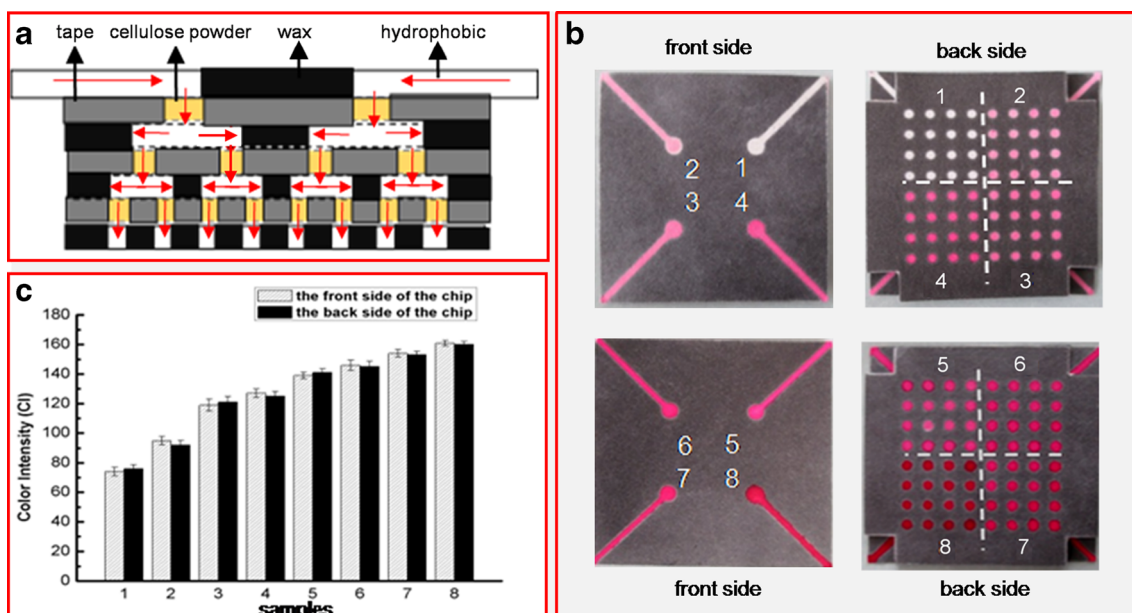
Cr (VI) assays that was critical for chromogenic reactions. The additional reagents that were in dry form, including basification reagents, masking reagents, and chromogenic reagents, were totally prestored in the different layer of the 3D paper microfluidics. When flows passed another layer by capillary wicking, they can combine with different reagents in different layers without mixing and finally reached into complex arrays of detection zones to bring about chromogenic results.

To assess the stability of the stored reagents, four heavy metal ions including Cu (II), Ni (II), Cd (II), and Cr (VI) was dissolved individually into ddH<sub>2</sub>O samples at a known concentration and assayed using 3D paper microfluidics first at initial time and then at 2, 4, and 6 months time points, respectively. The color intensity of each of the metal assays obtained at initial time was compared to the color intensity determined at 2, 4, and 6 months later (see Electronic Supplementary Material Fig. S2). The intensity levels obtained at these monitoring dates were generally similar and showed no significant statistical differences for all metal ions tested ( $p > 0.05$ ). It implied the paper chip's using life prepared using abovementioned formulas should be at least 6 months, which should be sufficient for practical application.

The cost of the materials used to make the paper chip in Fig. 1 was approximately \$3 of approximately \$0.7 per layer of paper and tape. For the identification of heavy metal, we used detection zones with 3-mm dimensions, so that the results of assays were easily visible by eye, and can be photographed accurately. A camera mobile phone, which was accessible to more and more people, was set as a low cost, portable, and easy-to-use detector to monitor the metal assay results on the paper-based chip. With the aid of professional image analysis software, the images of detection zones can be turned into specific values and evaluated to obtain the metal assay results. Meanwhile, these images can also be transmitted by e-mail or MMS messaging combined in the cell phone [26, 27] to the water quality monitoring station for further quantitative analysis, especially when something needed to be judged by an expert or large number of samples required testing. Advanced camera phones will combine personal digital assistant together and install the image analysis software directly in the cell phone to make it more powerful.

### Validation study of the 3D paper microfluidics

It is well known that heavy metals, including Cu (II), Ni (II), Cd (II), and Cr (VI), can show a certain color due to the reaction with the selective chromogenic reagent [28–31]. To assess whether the 3D paper microfluidics was able to detect parts per million levels of these metals, assays were conducted by dipping the paper chip into solutions



**Fig. 3** Flow running assay in 3D paper microfluidics. **a** Demonstration of fluids 3D moving in the paper chip. The hydrophobic wax patterned into the paper delimited channels through which the fluids moved laterally; the water-impermeable tape separated channels in neighboring layers of paper and holes punched into the tape enable vertical flow. Thus, the device extended paper-based chip from 1D lateral-flow system

to 3D system with complex microfluidic paths (red arrows in the presence of fluid moving directions). **b** Distribution each of eight different concentrations of amaranth into detection array in two separate paper chips. **c** The intensities of red in the inlet spot were compared with the average values of 16 zones in each region

containing levels of these metals. Electronic Supplementary Material Fig. S3 shows the plots of the color intensity obtained from separate experiments using varying concentration of Cu (II), Ni (II), Cd (II), and Cr (VI). In all cases, the data shows that increasing concentrations of the metal ions progressively increased the intensity of the color intensity. The standard curves were conducted within the linearity ranges, respectively, with the correlation coefficients  $\geq 0.9990$ , each. On the basis of these results, the calculated detection limits of Cu (II), Ni(II), Cd (II), and Cr (VI) were found to be 0.29, 0.33, 0.35, and 0.19 ppm, respectively.

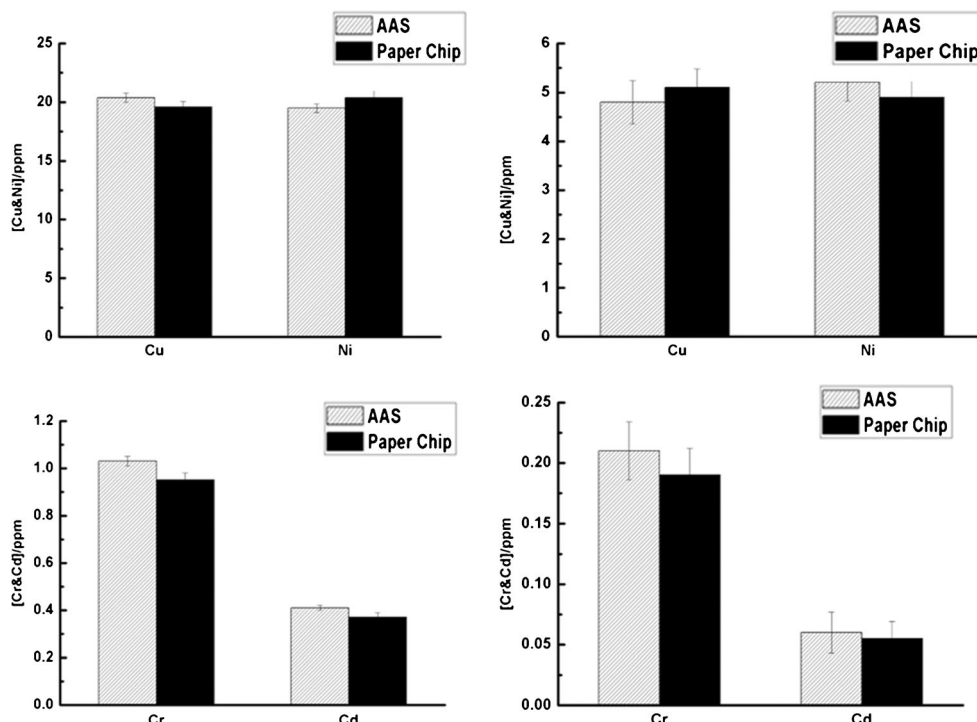
To further evaluate the quantitative performance of the 3D paper microfluidics, each of the metal ions was dissolved individually into ddH<sub>2</sub>O samples at two different known concentrations and assayed using both the 3D paper microfluidics and an AAS assay. The data obtained from AAS were compared to the concentration determined with 3D paper microfluidics using the standard curves shown in Electronic Supplementary Material Fig. S3. Figure 4 shows the comparison of Cu (II), Ni(II), Cd (II), and Cr (VI) values determined by the AAS method and 3D paper microfluidics assay. These two monitoring methods provided concentration levels that were generally within 10 % error for all metal ions tested, with the major advantages of low cost, high-throughput, rapid analysis time, and easy for user for 3D paper microfluidics.

#### Selectivity and interferences

To examine the ability to quantify heavy metal selectively, the colorimetric results by a combination of heavy metals were compared with results by each individual metal ion and were shown in Electronic Supplementary Material Fig. S4. The multiple color intensity from the mixture of metal assay in each detection zone was generally similar and showed no significant difference ( $p > 0.05$ ) to that from the individual metal assay in each corresponding detection zone. Meanwhile, absence of any of these metals resulted in no color change from the respective sensing zone. As expected, cross-sensitivity issues were not observed in all the detection zones, which indicated that the metal ions were able to be selectively qualified using the 3D paper microfluidics. Although it is possible that the indicator may respond to higher levels of the heavy metals we tested or other heavy metals not present in our assays, selective detection is possible if an appropriate amount or formula of masking reagents is utilized in the detection zones.

In order to evaluate the interferences from concomitant ions, a high concentration of NaCl (200 ppm) or KCl (100 ppm) was mixed with different concentrations of heavy metal ions and tested using the 3D paper microfluidics. Electronic Supplementary Material Fig. S5 shows that there was a negligible effect on the color intensities for Cu (II) in the presence NaCl or KCl; similar results were obtained for all other metal ions. Importantly, all the data were highly

**Fig. 4** AAs and 3D paper microfluidics assays of heavy metal ions spiked into ddH<sub>2</sub>O at different concentrations. Cu (II) (20 and 5 ppm), Ni(II) (20 and 5 ppm), Cd (II) (1 and 0.2 ppm), and Cr (VI) (0.4 and 0.1 ppm). Data are means of three determinations



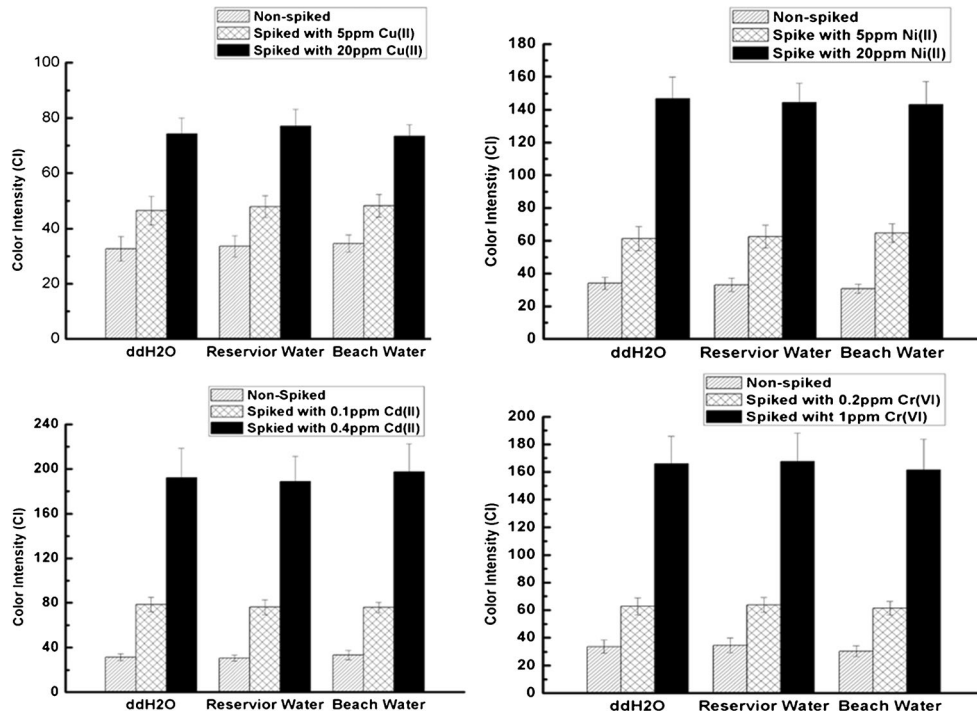
reproducible, showing the utility of the assay for monitoring heavy metals in environmental samples.

Testing of environmental samples

To demonstrate the applicability of the 3D paper microfluidics for the determination of heavy metals in real environmental

samples, distilled water (pH 5.5), reservoir water (pH 7.1, Biliu He Reservoir, Dalian, P. R. China), and beach water (pH 8.2, Baishi Bay, Dalian, P. R. China) were collected and mixed with known high and low levels of each metal ion. AAS analysis of the reservoir and sea water sample showed that no metals of interest were present at levels above 0.005 ppm, and all were at levels below their respective

**Fig. 5** Determination of Cu (II), Ni(II), Cd (II), and Cr (VI) spiked into ddH<sub>2</sub>O, reservoir water, and beach water, along with control data for the respective unspiked samples. Data are means of three measurements



detection limits for the paper chip. As shown in Fig. 5, unspiked samples resulted in no color change from the respective sensing zone, with the same color intensity as the control ( $p > 0.05$ ), demonstrating that all of the sample matrixes did not provide any impact on the 3D paper microfluidics. Environmental samples spiked with each of the heavy metal ions resulted in corresponding color change in each detection zone and provided concentration levels that were generally within 5 % error. Hence, it is possible to detect heavy metals in all of the sample matrixes, even with pH values varying from 5.5 to 8.2. These results demonstrate that the 3D paper microfluidics can be used for rapid analysis of either reservoir or beach water with no interferences.

In regions with limited resources, where the environmental monitoring infrastructure is less developed, many difficulties should be overcome by applying the low-cost device and ease of use must also be considered. In this case, paper-based device can be extremely useful. The developed 3D paper microfluidic device can perform multiplex metal assays in a simple and low-cost way, thus it has a significant advantage in monitoring of water quality, especially in developing countries.

## Conclusions

In this work, we developed a paper-based microfluidic device for colorimetric determination of selected heavy metals in water samples. Through simple combination of patterned paper by wax printing, tape and stacking, this device extended paper-based metal assays from simple 1D lateral-flow systems to 3D devices with complex microfluidic paths. Fluids can pass both vertically and laterally through multiple layer of paper, and can be distributed, combined with different reagents in different layer, and finally reached into complex arrays of detection zones to bring about chromogenic results. In the present prototype, four analyses simultaneously detecting in each the four metals and four replicates for each metal ( $4 \times 4 \times 4$ ) can be performed. The color intensity estimated by the software was related to the concentration of heavy metals, with the sensitivity to metals ions being  $\text{Cd (II)} > \text{Cu (II)} > \text{Ni (II)} > \text{Cr (VI)}$  and detection limits ranging from 0.19 to 0.35 ppm. This device retained full activity for at least 6 months at 25. The quantitative results of metal ions gained from 3D paper microfluidics were comparable to those obtained using a conventional AAS method but provide for a more rapid, easy for user, and low-cost detection platform. This device can identify individual metal ions from a mixture of metal ions, and showed no interferences from either nontoxic metal ions such as  $\text{Na(I)}$  or components found in reservoir or beach water.

In general, this 3D paper microfluidics associated with a camera mobile phone detector can be set as a low cost, high-

throughput, portable and easy-to-use platform for metal assays. With the flexible and easy patterning as well as the use of additional indicator/masking reagent/activation solution system, the current device might be expanded to a range of other metals and may provide an alternative method for identifying specific metal ions in mixtures with further tests.

**Acknowledgments** This work was supported by the National Natural Science Foundation of China (No. 41006071) and Scientific Research Project of Liaoning Education Department (No. L2013478 and L2013479).

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