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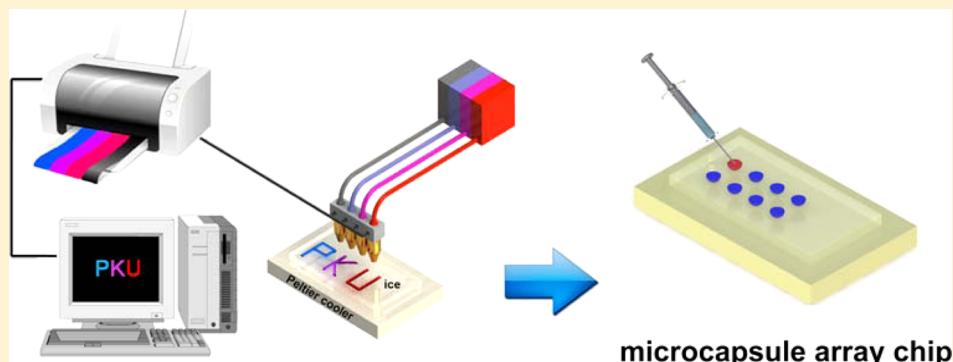
Portable, Easy-to-Operate, and Antifouling Microcapsule Array Chips Fabricated by 3D Ice Printing for Visual Target Detection

Hong-Ze Zhang,^{†,‡,§} Fang-Ting Zhang,^{†,§} Xiao-Hui Zhang,[†] Dong Huang,[‡] Ying-Lin Zhou,^{*,†} Zhi-Hong Li,^{*,‡} and Xin-Xiang Zhang^{*,†}

[†]Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry, Peking University, Beijing 100871, China

[‡]National Key Laboratory of Science and Technology on Micro/Nano Fabrication, Institute of Microelectronics, Peking University, Beijing 100871, China

Supporting Information



ABSTRACT: Herein, we proposed a portable, easy-to-operate, and antifouling microcapsule array chip for target detection. This prepackaged chip was fabricated by innovative and cost-effective 3D ice printing integrating with photopolymerization sealing which could eliminate complicated preparation of wet chemistry and effectively resist outside contaminants. Only a small volume of sample (2 μ L for each microcapsule) was consumed to fulfill the assay. All the reagents required for the analysis were stored in ice form within the microcapsule before use, which guaranteed the long-term stability of microcapsule array chips. Nitrite and glucose were chosen as models for proof of concept to achieve an instant quantitative detection by naked eyes without the need of external sophisticated instruments. The simplicity, low cost, and small sample consumption endowed ice-printing microcapsule array chips with potential commercial value in the fields of on-site environmental monitoring, medical diagnostics, and rapid high-throughput point-of-care quantitative assay.

Nowadays, paper-based microfluidic chips with low cost and portability have recently drawn significant attention for potential applications in biochemical analysis,^{1–3} immunoassays,^{4–6} food safety,^{7,8} and environmental monitoring,^{9,10} which especially are exploited as a multiplex point-of-care diagnostic device.¹¹ A promising diagnostic assay using paper-based microfluidic device fabricated by photolithography process was first reported by the Whitesides group.¹² Paper is an excellent substrate for making microfluidic with advantages of ubiquitousness, extremely low price, and good biocompatibility.¹³ Diverse 2D and 3D microfluidic channels have been fabricated on paper, which transports liquids only via capillary force in the predesigned pathways.¹³ Appealingly, paper-based microfluidic chips require small sample consumption, simple disposal, and minimal use of external equipment and a power source. However, samples on paper chips are exposed in open air without sterile conditions, thus dust and dirt may contaminate the assays or fragile reagents like enzymes will be prone to deactivation, lacking in protection. Besides,

turbidimetry allowing for a transparent substrate passed through by light is partly restricted on paper chips.¹⁴ Under these circumstances above, antifouling and transparent chips that still retain advantages of paper chips are highly desired.

In the other aspect of fabricating or replicating structures of chips, the dominant techniques for microchannel fabrication utilized in microfluidics are top-down methods divided to the main three categories, bulk/film-machining, surface-machining, and mold-machining.¹⁵ To date, these bulk/film-machining methods mainly create channels by etching trenches in the substrate wafer with weaknesses of using expensive photolithography, laser systems, and electron-beam lithography and requiring defect-free flat surfaces. In mold-machining, the mold in the inverse shape of the desired structure is usually made by producing a pattern (master) in a layer of photoresist on the

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surface of silicon wafer by photolithography or electron-beam lithography. Similarly, the fabrication of masters is rather expensive and time-consuming.

Distinguished from bulk/film-machining and mold-machining, surface-machining is relatively low-cost and easy to fabricate.¹⁶ In surface-machining, a bottom layer is deposited on the substrate wafer followed by deposition of the sacrificial layer and its patterning. Finally, the top layer is deposited on top of the sacrificial layer and patterned. The microchannel is formed by removing or etching the sacrificial layer. The conventional sacrificial materials like polysilicon¹⁷ and thermal-degradable materials¹⁸ often take too long time to remove.¹⁹ Compared with polymer materials, ice is contamination-free, low-temperature, biocompatible, and easily tractable and can be easily transformed reversibly between liquid state and solid state, which is an ideal sacrificial material based on phase changing for surface-machining.^{20,21} Meglinski et al.²² reported a rapid express modeling scaffold for tissue engineering in the ice (to replace high-cost biodegradable polymers) with a computer-guided CO₂ IR laser. Also Bossi et al.²³ developed an ice-based reconfigurable microfluidic system for separation and preconcentration of samples.

Compared with thin film deposition in surface-machining, soft lithography pours a liquid precursor of polymers like polydimethylsiloxane (PDMS) over the mold or the sacrificial layer and then liquid precursors are cured into the solid, which is quite simple to operate.²⁴ Besides, soft lithography has some remarkable advantages over other traditional forms of lithography such as photolithography and electron beam lithography, lower cost, wider substrate ranges especially nonflat surfaces, and no need for a photoreactive surface.²⁴

Inspired by surface-machining, soft lithography, and 3D printing technologies, we pioneered a soft fabrication method of microcapsule array chips by replacing thermoplastic materials in 3D printing with working aqueous solution as ink for ice printing and endowed ice sacrificial layers a fresh role as the indicating solution. Aqueous solution was printed onto the cold surface over the Peltier cooler and then frozen into an ice structure. More sophisticated 3D ice structures with fine feature sizes and even multilayered patterns can be achieved by processing the printing sequences designed by commonly used Microsoft Office software. On the basis of our proposed ice printing method, we successfully fabricated the microcapsule array chip as a significant supplement to paper chip in disposable use. Moreover, nitrite and glucose were chosen as model targets to accomplish a rapid visual quantitative detection. With the aid of automatic equipment, this kind of chip can be massively and cost-effectively produced and enables the evolution of highly efficient and versatile analytical tools in the biomedical field.

■ EXPERIMENTAL SECTION

Chemicals and Equipment. Horseradish peroxidase (HRP, E.C.1.11.1.7, ≥250 units/mg, RZ 3.0), glucose oxidase (GOx, E.C.1.1.3.4, type X-S, lyophilized powder, 130 units/mg, from *Aspergillus niger*), and D-(+)-glucose were obtained from Sigma-Aldrich (St. Louis, MO). N-(1-naphthyl)-ethylenediamine, sulfanilamide, and 4-aminoantipyrine (4-AAP) were purchased from J&K Chemicals (Beijing, China). Sodium nitrite (NaNO₂), sodium nitrate (NaNO₃), sodium sulfate (Na₂SO₄), sodium phosphate (Na₃PO₄), citric acid, D-(+)-fructose, D-(+)-mannose, sucrose, and phenol were purchased from Beijing Chemicals (Beijing, China). The

prepared buffer for glucose detection was phosphate buffer (PB, 0.1 M, pH 7.4). All samples and buffer solution were prepared using ultrapure water from a Milli-Q water purification system (Millipore).

The other equipment and materials we used were included as follows: four-color inkjet piezoelectric printer (EPSON, L111, Japan) and 3-pL Ultra Micro Dot print head (EPSON, Japan), Peltier cooler, digital thermometer and hygrometer, light cure medical adhesive (Loctite 3311), glass slide, polystyrene (PS) film, poly(methyl methacrylate) (PMMA), and Parafilm (Pechiney Plastic Packaging Company, Chicago, IL).

Colorimetric Assays. In this work, nitrite and glucose were chosen to ensure the fabricated microcapsule array chip was capable of being applied in quantitative analyses. The nitrite stock solution was prepared by dissolving sodium nitrite in ultrapure water. The nitrite indicating solution contains 50 mM sulfanilamide, 10 mM N-(1-naphthyl)-ethylenediamine, and 300 mM citric acid. A volume of 10 μL of nitrite indicator was sealed in each microcapsule of the array chip, then 2 μL nitrite stock solution was injected into the detection region with a microsyringe. The final color remained unchanged in 2 min before taking the photo image by Canon 700D digital camera (Japan).

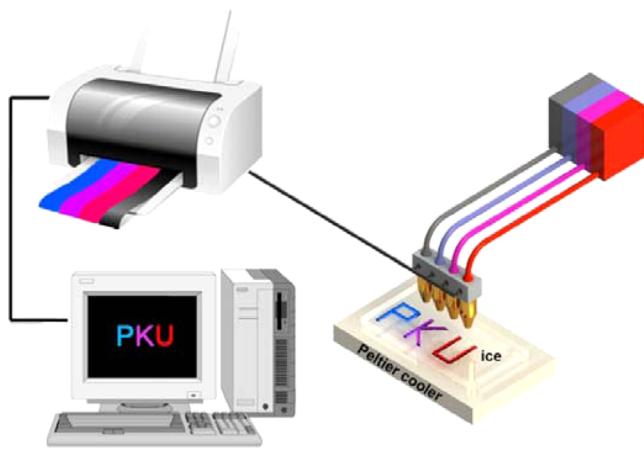
The glucose indicating solution contains 2.5 mM 4-AAP, 5 mM phenol, 100 μg/mL GOx, and 10 μg/mL HRP. A volume of 2 μL of glucose solution with different concentrations was applied to each microcapsule containing 10 μL of glucose indicator. Then the photo image was taken until the color was constant.

Image Processing. To quantify the color response, a commercially available digital camera (Canon, EOS 700D) was utilized to record the visual readouts of colorimetric assays. Then the photos were analyzed by ImageJ which is a public domain Java image processing program (<http://imagej.nih.gov/ij/>). ImageJ gives the mean gray value of each detection region weighing the color intensity to quantify under the same conditions.

■ RESULTS AND DISCUSSION

3D Fabrication by Ice Printing. Herein, an innovative and cost-effective method was proposed to fabricate the 3D structure featured by ice printing. Different from 3D printing with polymers, 3D ice printing introduced water solution as ink. Aqueous solution was printed onto the cold surface under the ice point over the Peltier cooler and then frozen into the ice structure layer by layer. Repeating the step by the automatic nozzle, a more complicated 3D ice structure with a fine feature size can be constructed. The prototype of the ice printing system modified by a commercially available 4-color inkjet piezoelectric printer with 90 × 2 micronozzles is shown in Scheme 1. A Peltier cooler and a temperature controller were used to maintain the low temperature (lowest -30 °C). The whole system was placed inside a glovebox filled with pure nitrogen isolated from the outside. Meanwhile, dry reagent was utilized to obtain a dry atmosphere to avoid frosting on the cold surface. The inside environment was monitored by a digital thermometer and hygrometer.

The design of the ice structures is flexible with respect to geometry, size, and height. The pattern for ice printing similar to printing on paper was designed via Microsoft Office software. The thinnest line of 100 μm and droplet smaller than 20 μm in diameter can be obtained by means of the 3-pL Ultra Micro Dot print head of Epson. A mechanical x-y stage

Scheme 1. Conceptual View of Ice Printing System

guarantees an accuracy with $\pm 5 \mu\text{m}$, which can be improved to $1 \mu\text{m}$ using the subfemtoliter method. As demonstrated in Figure 1a, a 10×10 ice pillar array was printed on a silicon substrate. Figure 1b shows an ice micropillar array with different heights ranging from $150 \mu\text{m}$ to 2 mm on silicon with a pillar size of $400 \mu\text{m} \times 300 \mu\text{m}$ by controlling the printing cycles (e.g., 210 cycles for 2 mm height). Furthermore, multilayered mesas with diverse reagents also can be achieved by designing the printing process. Figure 1c presents double-layered mesas with the first colorless layer and the second dyed layer in different sizes.

Moreover, combining with soft lithography which utilizes a light cure medical adhesive for sealing, 3D ice printing technology can guide a new evolution direction of drug-delivery microdevices. In conventional microfabrication, drugs are injected into the assembled reservoir individually,²⁵ which is not convenient to operate and thus limits mass production of

drug-delivery microdevices. With the aid of ice printing, drug solution can be directly printed as a reservoir and then packaged instantly which can greatly simplify the manufacturing process of drug-delivery microdevices.

Fabrication of Microcapsule Array Chip. On the basis of our proposed ice printing method, we successfully fabricated the microcapsule array chip for target detection. Figure 2a

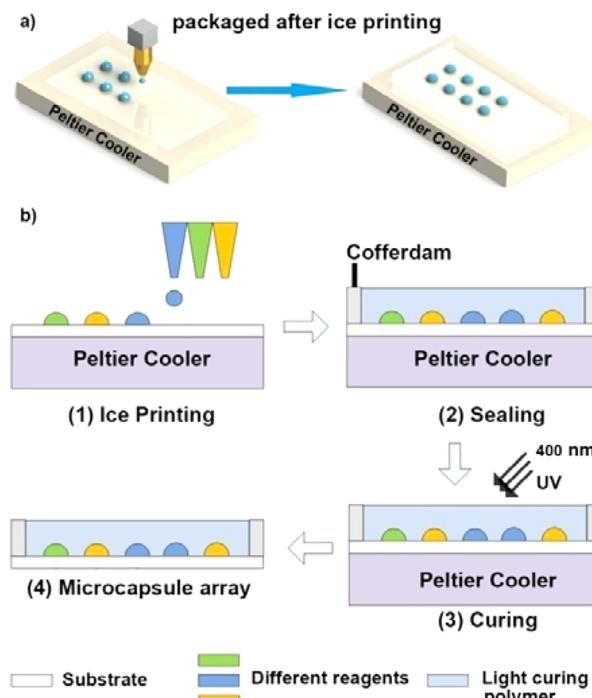


Figure 2. (a) Schematic view of the ice printing method and (b) the fabrication process of the multitarget microcapsule array.

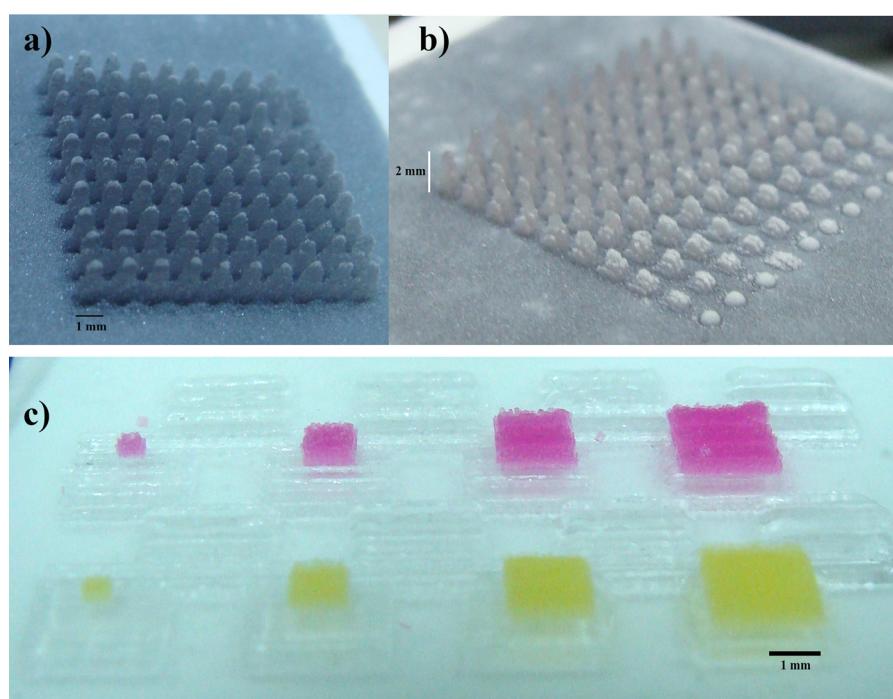


Figure 1. Photographs of diverse ice structures: (a) a 10×10 ice pillar array on silicon, (b) an ice micropillar array with different heights, and (c) double-layered mesas.

shows the schematic view of the manufacture of microcapsule array chips using the ice printing method. Figure 2b elaborates on the detailed process. The substrate was placed on a Peltier cooler, and then a fixed small-volume liquid droplet could be printed separately from the nozzle and frozen into solid ice as soon as contacting the cold surface below the ice point. Ice beads were sealed by the photopolymer without melting. Then the chip was prepackaged with light curing medical adhesive which was restricted by the PMMA cofferdam and solidified by the 400 nm light source (light emitting diode, LED). The encapsulated array chip was well sealed for long-term preservation with the advantages of reducing the evaporation of solution and resisting contaminants. All fabricated chips can be stored below 0 °C and used at room temperature with a phase transition from ice to water in 1–2 min. In particular, by sealing different reagents for different targets on the same microcapsule array chip, we can also accomplish multitarget detection.

Selection of Substrate for Chips. Theoretically, ice printing can be used on a variety of substrates (such as glass, metal, silicon and polymer film) and geometries. More accurately, any substrate with good thermal conductivity is valid for ice printing. The temperature of Peltier cooler should be set according to thickness and thermal conductivity of different substrates. To guarantee the fine shape of divided microcapsule, three kinds of substrates, namely glass slide, polystyrene (PS) film and Parafilm which are easily available and low-cost were tested by dropping 10 μL of dyed solution onto them. Finally, we chose a transparent and hydrophobic polystyrene (PS) film with the thickness of 50 μm as the optimal substrate. The reagent droplets have a higher contact angle on the PS film than that on the hydrophilic glass slide, which benefits the regular pattern of microcapsules, as shown in Figure 3. Compared with Parafilm which has similar hydro-



Figure 3. Dyed detection reagent droplets (10 μL) on three different substrates.

phobicity, PS has also been widely used as the material of biochemical containers, such as Petri dishes and ELISA plates, which enables its wide application.

Nitrite and Glucose Assays. To verify the feasibility of quantitative biochemical analyses using microcapsule array chips, we selected nitrite and glucose for models as proofs of concept. Nitrite and glucose assays are both based on the most used colorimetric mechanisms. The colorimetric determination of nitrite was based on the fundamental reaction of diazotization and coupling (i.e., Griess reaction) involving nitrite, *N*-(1-naphthyl)-ethylenediamine, and sulfanilamide²⁶ as shown in Figure 4a, which reacts completely and instantaneously and gives a final purple color that remains constant for several hours. The glucose assay is based on a color change test resulting from the Trinder reaction²⁷ in Figure 4b, which is comprised of 4-aminoantipyrine and phenol and hydrogen peroxide (H_2O_2) to form a red-colored quinoneimine, catalyzed by HRP. H_2O_2 is produced by an initial reaction where glucose is oxidized in the presence of GOx into H_2O_2 and gluconic acid.

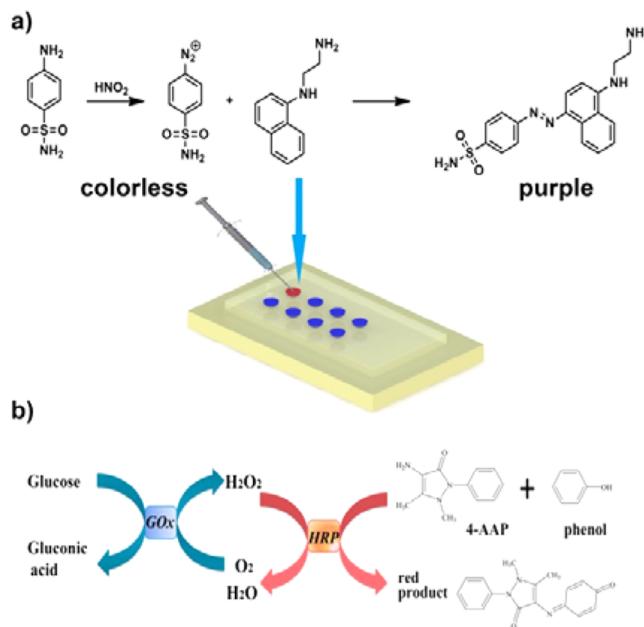


Figure 4. Reaction mechanism of the microcapsule array for the (a) nitrite assay and (b) glucose assay.

Indicating reagent with 10 μL was packaged in a divided microcapsule detection region. As small as 2 μL of sample solution was introduced into each microcapsule then ignited a chemical or an enzymatic reaction and yielded a color change from colorless. Then the images were analyzed by ImageJ software by calculating the mean gray value after being converted to gray scale. An instant detection by the naked eye was achieved without external instruments except for one microsyringe for injecting the samples. Compared with traditional spectrophotometric methods, microcapsule array chips address the drawbacks such as large sample consumption and instrumental needs.

Nitrite has been proposed as a potential marker for many human health conditions such as periodontal disease.²⁸ In Figure 5a, the image of the nitrite assay distinctly presents different purple color responses to samples with different concentrations. The color responses in the detection regions were also reasonably uniform, which suggested the reliable performance of the microcapsule array. The calibration curve is shown with a concentration range from 0.1 to 7.5 mM. Furthermore, we confirmed the specificity of colorimetric reagents by replacing nitrite ion with other ions including nitrate ion, sulfate ion, and phosphate ion as shown in Figure S1 in the Supporting Information. The stability of the microcapsule array chip for nitrite was also examined in 20 days as shown in Figure S2 in the Supporting Information.

Glucose was chosen since the monitoring of blood glucose levels is crucial in the clinic. The color images of the tested chip is shown in Figure 5b. The calibration curve shows an obvious discrimination between 5 mM (normal level) and 8 mM (blood glucose above 7.8 mM indicates a prediabetic state of hyperglycemia, which means a state of increased risk of progressing to diabetes according to the World Health Organization.²⁹). The specificity of GOx toward glucose was confirmed by replacing the substrate with fructose, mannose, and sucrose in Figure S3 in the Supporting Information. The stability of microcapsule array chip for glucose detection was

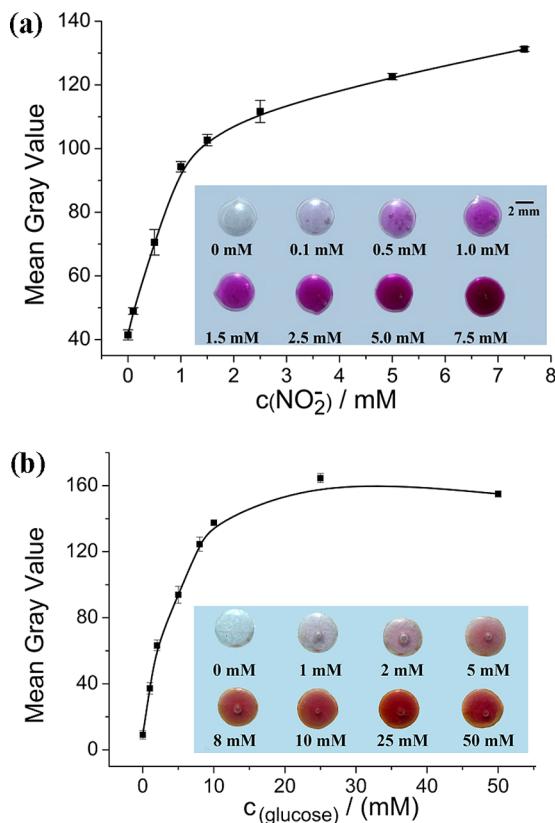


Figure 5. Calibration curve for (a) nitrite and (b) glucose detection using image processing software ImageJ for color quantification.

also examined in 20 days as shown in Figure S4 in the Supporting Information.

CONCLUSION

We proposed a low-cost, portable, and miniaturized microcapsule array chip fabricated by innovative and cost-effective 3D ice printing method for rapid visual detection taking nitrite and glucose as model targets. 3D ice printing utilized a commercially available printer and commonly used Microsoft Office, which facilitated its promotion. A variety of ice structures with different geometries, sizes, and heights, even multilayered ones, can be easily achieved. Sealed by noninvasive light cure adhesive in ice form, the microcapsule array chips could be preserved for long-term and effectively resist outside contaminants. Particularly, multitarget analysis can be realized by packaging different indicating reagents for different targets on the same microcapsule array chip. Moreover, a smartphone-based analytical application can be designed to process color images of tested chips by calculating the mean gray value and output results to users immediately. The ice-based microcapsule array chips might have the potential to be applied in on-site primary diagnostic tests, environmental monitoring, and a food safety survey. Furthermore, it can be developed to point-of-care platforms allowing for in-home determinations without a trained specialist.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01440.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: zhoul@pku.edu.cn. Phone: +86-10-62754112. Fax: +86-10-62754112.

*E-mail: zhhl@pku.edu.cn. Phone: +86-10-62766581. Fax: +86-10-62751789.

*E-mail: zxz@pku.edu.cn. Phone: +86-10-62754680. Fax: +86-10-62754680.

Author Contributions

[§]Hong-Ze Zhang and Fang-Ting Zhang contributed equally to this work.

Notes

The authors declare no competing financial interest.

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