Supporting Information

An Ink-jet Printing Strategy for Extensive Exploration of One Chemical Action with Three Interactive Variations

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Experimental

Screening

For screening three suitable chemical reagents for this study, 15 reagents were added and reacted with amino acid and ninhydrin, heated in an oven at 100°C for 5 minutes. The results were recorded and analyzed by a CCD camera and computer, and presented by means of math method, RGB color-space, chromatic aberration was calculated by color difference formulas (Fig. S1).

Reagents and Material

Distilled water was adopted throughout this study for preparing all the experimental reagents, they were A(NiSO₄), B(Na₂S₂O₃), C(C₄H₆O₆, tartaric acid) and ninhydrin (C₉H₄O₃). The concentrations were NiSO₄ 0.2 mol/L, Na₂S₂O₃ 0.1 mol/L, C₄H₆O₆ 0.1 mol/L, ninhydrin 2wt%. Surface tension of experimental reagent was fixed as the same with printing ink in experimental preparation.

For the experimental sample, red grape (it is fresh and contains large amounts of amino acids) was squeezed and filtered for its supernatant.

Designing template and creating reaction chip

Printing template was designed by Photoshop (Fig. 1a). The template was divided into four areas and marked as I, II, III, IV, expressed as an assembled pattern by the colors of three inks C, M, Y. The color represented the kinds of ink, and the color depth represented the amount of ink. The top left corner of template was marked I, top right corner was II, bottom left corner was III, and bottom right corner was IV. Three parts of I, II, III consisted of two kinds of inks, C and M, C and Y, M and Y respectively, part IV consisted of three inks C, M,Y. The amount of

each ink changed both continuously and gradually. Finally, the printing template has been designed and completed. On the template, there were a large number of micro-units that were formed by cross combinations of the kinds and the amounts of three inks.

Create reaction chip. Firstly, three kinds of ink were replaced with three chemical reagents A (NiSO₄), B (Na₂S₂O₄), C (C₄H₆O₆, tartaric acid) in ink boxes in experiment, then the reagents were output on a blank substrate (medical-PET film) according to the designed template. Secondly, ninhydrin reagent was sprayed evenly on the substrate. Last, the lots of micro-reaction units (combinations of the kinds and the amounts of three reagents in four reaction areas) on the substrate were completed, the substrate was named reaction chip (Fig. 1b). Every micro-reaction unit was a micro-reactor.

Experimental reacting

For sample reacting with ninhydrin on the reaction chip, fresh red grape was squeezed and filtered for its supernatant. Portable ultrasonic nebulizer was utilized to atomize supernatant to make it become tiny droplets, and then tiny droplets were transported, falling evenly on the reaction chip. Last, the chip was placed into an oven at 100° for 5 minutes and taken out.

Digitization of experimental results

After reacting, the information of experimental results was demonstrated as a pattern on the substrate, and then recorded by a CCD camera. For clearly analyzing information, the results were quantified in digital form. The main procedures were as follows: (1) Pre-processing. The images of experimental results were processed by unifying light intensity of background, rotating to make them be consistent. Four reaction areas were cut off and marked as I , II ,III,IV, respectively, according to designed template. (2) Digitization. The colors on images were converted into digital signals and presented as a gray image (Fig. 2b). All the gray values of

four reaction areas were obtained by a software.

Information analysis of template

In Fig. S2, the rules on amount of the three kinds of ink on the design template were studied at arbitrary positions (pixels of rows and columns). The amounts of all inks changes both continuously and gradually. In the parts of I, II, III(a-c), the amount of one ink continuously decreases from top to bottom, and another decreases from left to right. In part IV(d), the amount of C continuously decreases from top to bottom, the M amount decreases from left to right, the Y amount continuously decreases toward the center from top and bottom. The amounts of C, M, Y decreases at 100%-0%. In experiment, the combined regulation of the three chemical reagents (they replaced inks) are corresponded to inks'.

Error analysis

Error of experimental results also was analyzed. The gray values of two parallel samples were calculated by relative standard deviation and expressed in Fig. S3. The amount of micro-reaction units among parallel samples reaches to 90%, relative standard deviations of these units are less than 5%, and relative standard deviations are less than 10% at 99% units. The resulting error between parallel samples is small; the repetition and operation ability are in the new method.



Fig. S1 Progress of screening experiment.

$$D(x_i, x_j) = \sqrt{(r_i - r_j)^2 + (g_i - g_j)^2 + (b_i - b_j)^2}$$
, moreover, $x_i = (r_i, g_i, b_i)$, $x_j = (r_j, g_j, b_j)$



Fig. S2 Change rules of the reagents amount on the designed template. (a) C: 198th column;
M: 395th row; (b) C: 198th column; Y: 395th row; (c) M: 198th column; Y: 395th row; (d) C, Y: 294th column; M: 99th row.



Fig. S3 Error analysis of the experiment results.



Fig. S4 Changes of the gray values. (a) The gray values in reaction I under the condition of A and B, A amount continuously decreases along the lateral displacement. (b) The gray values in reaction III under the condition of B and C, B amount continuously decreases along the lateral displacement. (c) The gray values in reaction II under the condition of A and C, C amount continuously decreases along the lateral displacement.



Fig. S5 Images of the control experiments. (a) Ninhydrin reaction in the absence of the amino acid sample. (b) Ninhydrin reaction in the absence of the three chemical reagents.