Supplementary Information

Portable detection of multiple mycotoxins based on sonic toothbrush, microfluidic chip and smartphone

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Experimental details

S1. Materials and reagents

All DNA strands (Table S1) were synthesized and purified in Sangon Biotech. Co., Ltd (Shanghai, China). Streptavidin-alkaline phosphatase and disodium p-nitrophenyl phosphate (pNPP) were purchased from Sangon Biotech. Co., Ltd (Shanghai, China). Dopamine was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ochratoxin A (OTA), aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), gliotoxin (GT), aflatoxin B_2 (AFB_2), and aflatoxin M_1 (AFM_1) were purchased from Pribolab. Co., Ltd. (Qingdao, China). The corn powder quality control products of OTA, AFB_1 and FB_1 were purchased from Clover Technology Group Inc. (Manufacturer: Trilogy, USA). The negative corn powder was purchased from Beijing Meizheng Biotechnology Co., Ltd (Beijing, China). All solutions were prepared and diluted with ultrapure water (18.2 MΩ·cm). The 1 mm polymethyl methacrylate (PMMA) plates were purchased from Xinbao Plastic Industry (Taobao, China, https://m.tb.cn/h.f8z1Ctq?sm=609e8f). The 0.14 mm silicone films were purchased from Hongtian Window Film Factory Store (Taobao, China, https://m.tb.cn/h.fjD6Wsr?sm=eb6a58).

Table S1
Sequences of DNA used.

Name	Sequence (from 5' to 3')
Aptamer-OTA*	Biotin-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA
cDNA-OTA	H_2N-T_{10} TGT CCG ATG CTC
Aptamer-AFB_1*	Biotin-AGT TGG GCA CGT GTT GTC TCT CTG TGT CTC GTG CCC TTC GCT AGG CCC ACA
cDNA-AFB_1	H_2N-T_{10} TGT GGG CCT AGC GA
Aptamer-FB_1*	Biotin-ATA CCA GCT TAT TCA ATT AAT CGC ATT ACC TTA TAC CAG CTT ATT CAA TTA CGT CTG CAC ATA CCA GCT TAT TCA ATT AGA TAG TAA GTG CAA TCT
cDNA-FB_1	H_2N-T_{10} AGA TTG CAC TTA CTA

*The aptamer sequences of the three mycotoxins were designed according to the reference[1].

S2. Preparation of solutions

S2.1 HEPES buffer (10 mM, pH = 7.0, 120 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$)

Added 0.5958 g HEPES, 1.7532 g NaCl, 0.0932 g KCl, 0.2541 g $MgCl_2 \cdot 6H_2O$ to 200 mL water and used NaOH to adjust the solution to pH 8.5, then added water to a final volume of 250 mL. The buffer was filtered by a 0.22 μm sterile filter and stored at -20 °C.

S2.2 Tris-HCl (10 mM, pH=8.5)

Added 0.3028 g Tris to 200 mL water and used HCl to adjust the solution to pH 8.5, then added water to a final volume of 250 mL. The buffer was filtered by a 0.22 μm sterile filter and stored at -20 °C.

S2.3 Tris-HCl (100 mM, pH=9.5, 5 mM $MgCl_2$)

Added 3.0275 g Tris, 0.2541 g $MgCl_2 \cdot 6H_2O$ to 200 mL water and used HCl to adjust the solution to pH 9.5, then added water to a final volume of 250 mL. The buffer was filtered by a 0.22 μm sterile filter and stored at -20 °C.

S2.4 1 μM Aptamer-OTA, Aptamer-AFB_1, Aptamer-FB_1, cDNA-OTA, cDNA-AFB_1, cDNA-FB_1

The dry powder of the six DNA strands was respectively dissolved by a certain amount of sterile water according to the instructions to obtain 100 μM solutions, then diluted to 1 μM with HEPES buffer and stored at 4 °C.

S2.5 0.2 U/mL streptavidin-alkaline phosphatase

According to the instructions, 600 U/mL streptavidin-alkaline phosphatase was diluted to 0.2 U/mL with HEPES buffer and stored at 4 ℃.

S2.6 pNPP solution

A certain amount of pNPP was dissolved by 100 mM Tris-HCl buffer. The pNPP solution should be prepared fresh before the experiment.

S2.7 The mixed substrate of pNPP and methylene blue

First, 10 mg methylene blue was added to 10 mL sterile water to obtain 1 mg/mL methylene blue, then the solution was diluted with pNPP solution to obtain a mixed substrate. The mixed substrate should be prepared fresh before the experiment.

S2.8 Negative sample solution

1.0 g negative corn powder was added to 4 mL 80% methanol and oscillated for 3 minutes, then the solution was filtered by a 0.22 μm sterile filter. 2 mL of the filtrate was diluted by 8 mL HEPES buffer and stored at 4 ℃.

S2.9 Positive sample solution

0.1 mL 1 μg/mL OTA, AFB_1, and FB_1 (dissolved in methanol) were added to 10 mL negative sample solution. The final solution contained 10 ng/mL OTA, 10 ng/mL AFB_1, and 10 ng/mL FB_1, and was stored at 4 ℃.

S2.10 Mycotoxin sample solution

A certain concentration of OTA, AFB_1 and FB_1 was added to the negative sample solution to obtain the mycotoxin sample solution.

S3. Design and manufacture of an extraction device

To fix a 1.5 mL centrifugal tube to the sonic toothbrush (Philips, Holland), a centrifugal tube adapter (Fig. S1) was designed by SolidWorks and fabricated by a 3D printer (Raised 3D Pro 2, Shanghai Fuzhi Information Technology Co., Ltd., China).

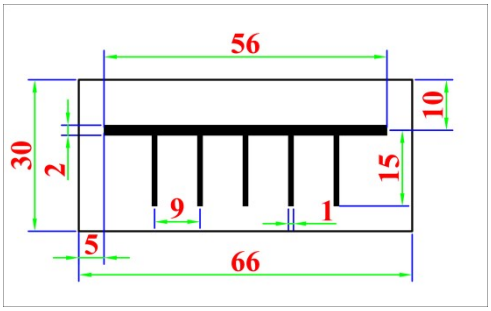
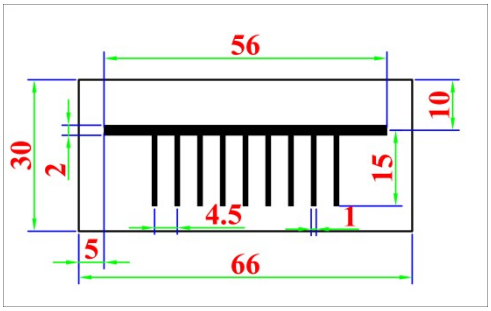
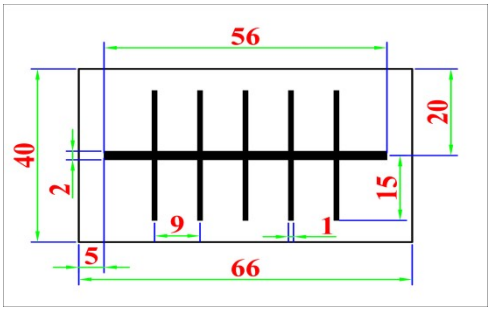
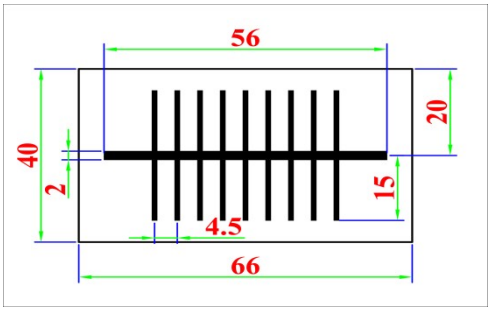
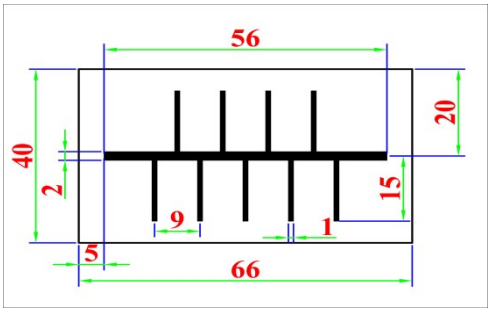
Fig.S1 Sonic vibration extraction device

S4. Design and fabrication of chip

In order to investigate the influence of chip structure, we designed chips with different structures. The detailed structures and dimensions were listed in Table S2. Compared with our previous report[2], the fabrication procedure of the chip was simplified. The chip was directly bonded with the adhesive transparent silicone film without double-sided adhesive (Fig. S2).

Table S2

Chip structures and sizes.

 S-5	 S-9
 D-10	 D-18
 D-9	---

These chips consist of one central channel (56 mm × 2 mm × 1 mm) and several branch channels (15 mm × 1 mm × 1 mm), named S-5, S-9, D-9, D-10, and D-18 respectively according to the distribution types (“S” for single-sided and “D” for double-sided) and the number of branch channels. The overall size of S-5 and S-9 chips was 66 mm × 30 mm × 1.28 mm, and that of D-9, D-10, and D-18 chips was 66 mm × 40 mm × 1.28 mm.

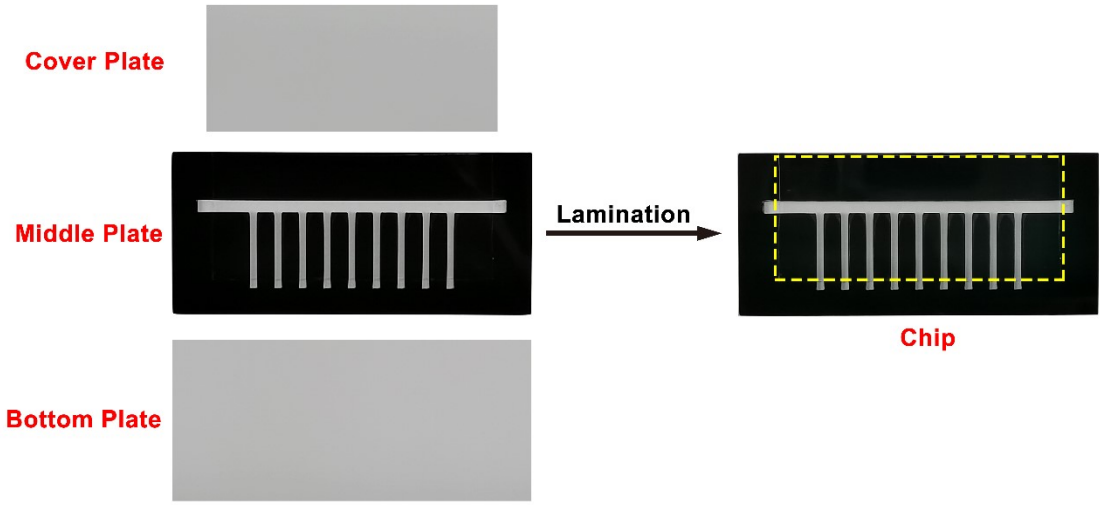


Fig. S2 Fabrication of chips. S-9 chip was chosen as an example. The cover plate and bottom plate were silicone film with adhesive, middle plate was PMMA with channels. The cover plate, middle plate and bottom plate were bonded to form a complete chip. A laser cutting machine (Han's Laser CMA0604-CO2-30) was used to cut plates and carve channels. The yellow dotted line was the location of the cover plate, and the size of the cover plate was smaller than the area of the channels, to leave the inlet and outlet.

S5. Modification and characterization of polydopamine on chips

To make chip channels hydrophilic and easy to immobilize aptamer probes, the channel surface was modified with polydopamine. Dopamine can undergo autoxidation reaction in air to form polydopamine under alkaline conditions.[3] The chip was modified with polydopamine according to the reference[4].

First, 2 mg/mL dopamine (10 mM Tris-HCl buffer, pH 8.5) was added into chips and incubated overnight at room temperature. Then the chips were washed with ultrapure water and dried. Finally, Drop Shape Analyzer (KRUSS GmbH, Hamburg, Germany) was used to measure the contact angles of the silicone films on chips before and after polydopamine modification. As shown in Fig. S3, the contact angles of the silicone film before and after polydopamine modification were 103° and 36° respectively, indicating that the polydopamine-modified chip was high-hydrophilic.

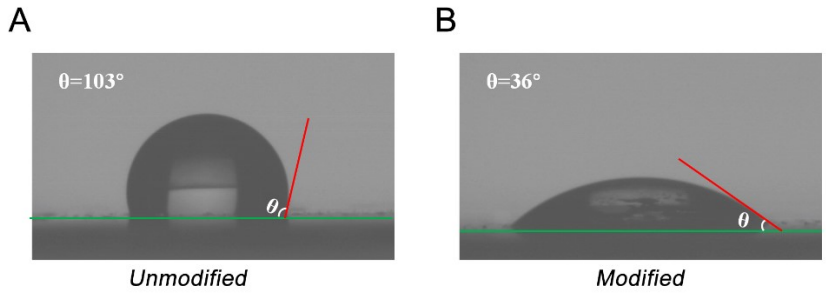


Fig. S3 The contact angles of silicone film before and after polydopamine modification.

S6. Investigation of the distribution of solution in chips

One of the key points for multiple mycotoxins detection on the chips was to divide the solution equally into multiple parts. Therefore, we investigated the distribution effect of the solution in the chips modified with polydopamine. 0.05 mg/mL methine blue (MB) solution was used as the indicator solution. As shown in Fig. S4, when the MB solution was loaded into central channels on the chips, all channels were filled with the blue solution through capillary action. When filter paper contacted the end of the central channels, the solution in central channels was rapidly sucked, while the solution in branch channels was retained. It was because of the smaller size and larger fluid resistance in branch channels, as well as the vertical fluid direction between the central channel and branch channels.[2,5] The above results showed that all chips were able to distribute solution as expected. Therefore, the chips with appropriate structures could be selected according to the species of analytes.

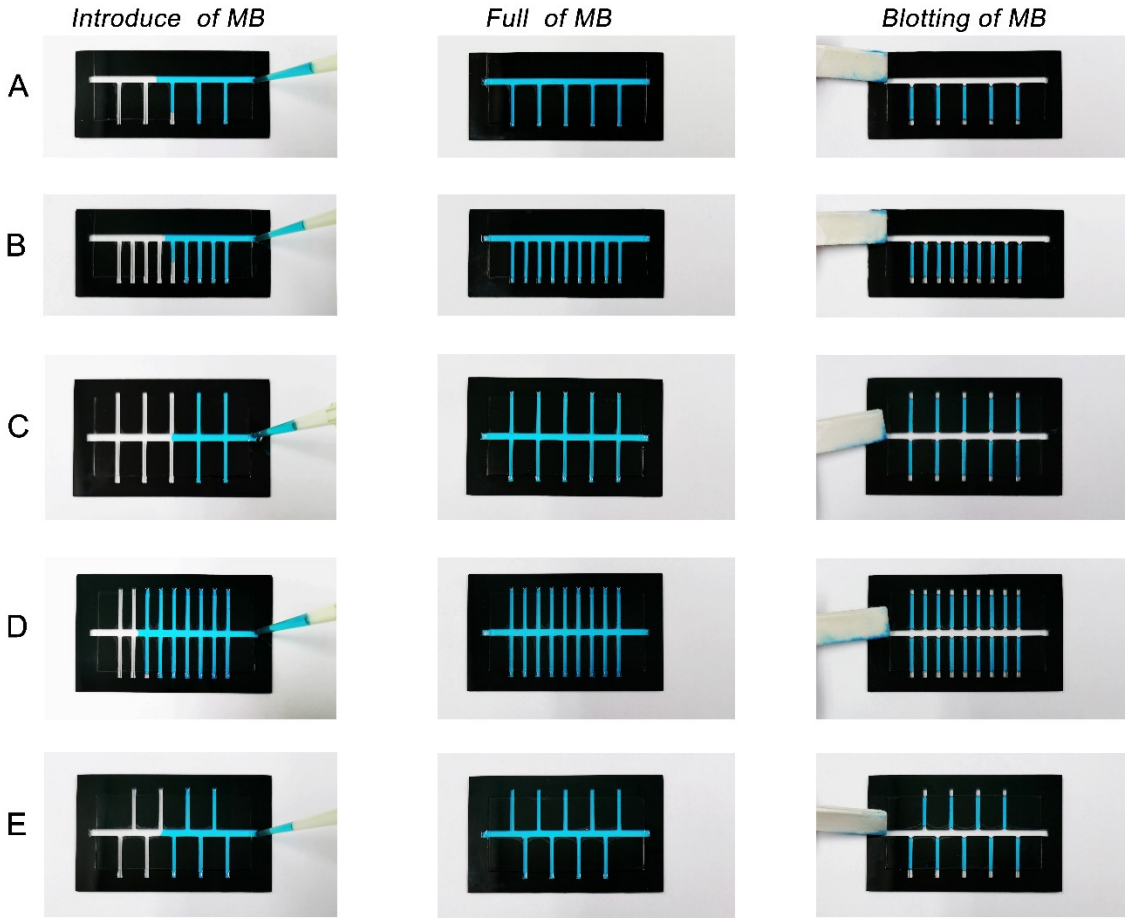


Fig. S4 The solution distribution in chips.

S7. Design and fabrication of a dark box

To eliminate the interference of ambient light, a dark box (Fig. S5) was designed by SolidWorks and fabricated by a 3D printer. The dark box was composed of three parts: the main body, the adapter for the smartphone, and the slider for chips. To provide a stable light source, a LED was installed on the dark box. To keep out external light, the dark box was covered with black film.

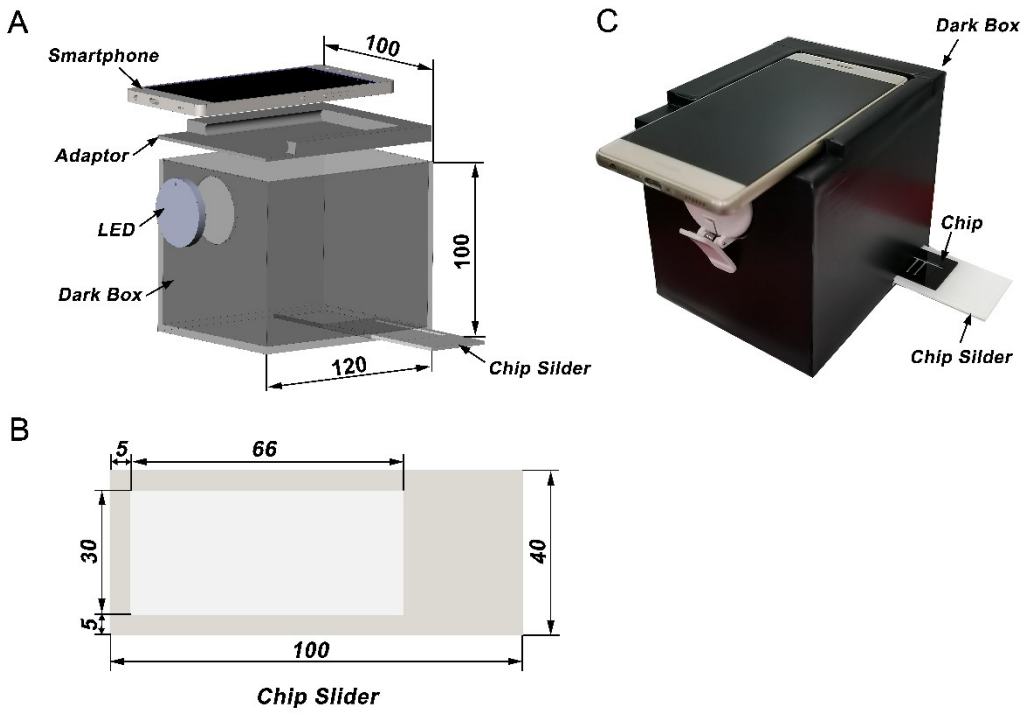


Fig. S5 Design and fabrication of the dark box. (A) The structure and size of the dark box. (B) The structure and size of the chip slider. (C) The photograph of the dark box. (Unit: mm).

S8. Signal acquisition and processing based on a smartphone

The RGB pick App "Color Grab" and the software development App "C++ Compiler" were downloaded and installed from the Android App Store. The "Color Grab" was used to acquire the color signals (G/B, ratio of green channel to blue channel of RGB) during target detection. The "C++ Compiler" was used to develop the data processing App "Sens-Mycotoxins" to calculate the concentrations of mycotoxins based on to the linear fitting equations for OTA, AFB_1, and FB_1:

```
#include<stdio.h>
int main ()
{
float G1, B1, G2, B2, G3, B3, Y1, Y2, Y3, C1, C2, C3;
printf ("Please enter values of G(OTA), B(OTA), G(AFB1), B(AFB1), G(FB1), B(FB1) (Separated by spaces):\n");
scanf ("%f %f %f %f %f %f", &G1, &B1, &G2, &B2, &G3, &B3);
Y1=G1/B1;
Y2=G2/B2;
Y3=G3/B3;
if(Y1<1.41) printf ("Please measure OTA after dilution.\n");
else {C1=(2.82-Y1)/2.82;
if (C1<0) printf ("OTA measure error, please re-measure.\n");
else printf ("Concentration of OTA is %.2f ng/mL.\n", C1);};
if(Y2<1.45) printf ("Please measure AFB1 after dilution.\n");
else {C2=(2.84-Y2)/1.39;
if (C2<0) printf ("AFB1 measure error, please re-measure.\n");
else printf ("Concentration of AFB1 is %.2f ng/mL.\n", C2);};
if(Y3<1.69) printf ("Please measure FB1 after dilution.");
else {C3=(2.84-Y3)/0.23;
if (C3<0) printf ("FB1 measure error, please re-measure.");
else printf ("Concentration of FB1 is %.2f ng/mL.", C3);}
}
```

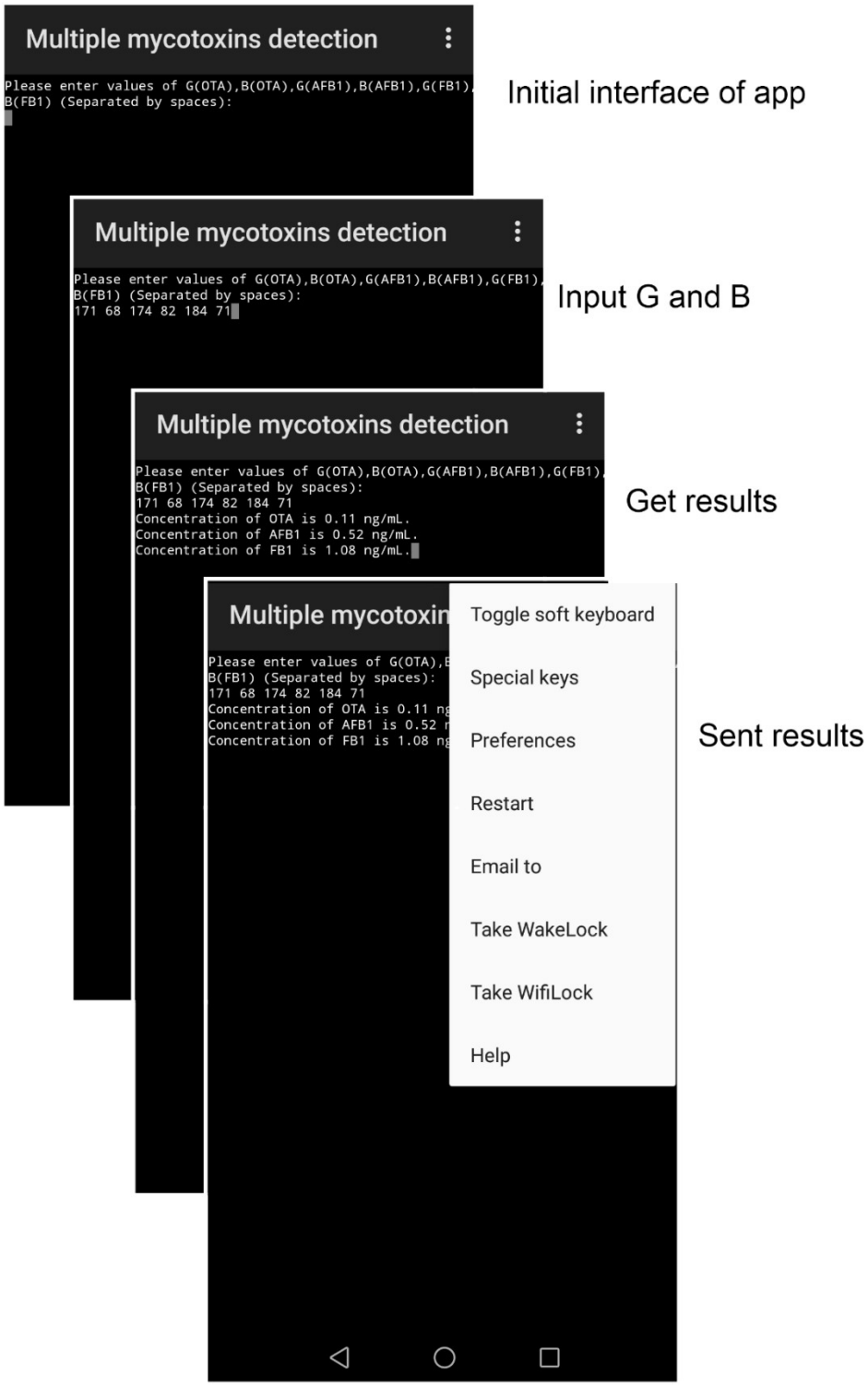


Fig. S6 Signal processing of mycotoxins detection through the "Sens-Mycotoxins" App. G and B values were input into the App, then the detecting results could be obtained.

S9. Simultaneous detection of three mycotoxins on chip

S-9 chip was used for the simultaneous detection of three mycotoxins. In chip washing step, as shown in Fig.S4, the washing buffer was added from one end of the main channel and filled the chip channel. Then, the solution in the main channel was blotted out from the other end, and the solution in each branch channel was blotted out from the end of the branch channels. The solution in channels could be rapidly sucked with filter paper at the end of the channels. Each washing step should be carried out five times.

S9.1 Probe modification.

On the surface of silicone chip, dopamine can undergo autoxidation reaction in air to form polydopamine under alkaline conditions.[3] The amino group on the DNA sequence can react with the carbonyl group on polydopamine to form a Schiff base for binding aptamer probe to the chip surface.[6] The covalent linkage was stable under neutral and weak alkaline conditions.

First, 1 μM aptamer of three mycotoxins and 1 μM corresponding cDNA were hybridized for 2 hours to obtain the OTA probe, AFB_1 probe, and FB_1 probe. Then, the OTA probe was added to branch channel 1, 2, and 3 of the polydopamine-modified chip, the AFB_1 probe was added to the branch channel 4, 5, and 6, and the FB_1 probe was added to branch channel 7, 8, and 9. The chip was washed with HEPES buffer after incubation for 3 hours at room temperature. Finally, 1 mg/mL BSA (HEPES buffer) was added to the chip, which was washed with HEPES buffer after blocking for 2 hours at room temperature.

S9.2 Detection of mycotoxins.

First, negative sample solution was added to branch channel 1, 4, and 7 of the chip modified with probes. Positive sample solution was added to branch channel 2, 5, and 8. The sample solution was added to branch channel 3, 6, and 9. The chip was washed with HEPES buffer after incubation for a period at room temperature. Then, 0.2 U/mL streptavidin-alkaline phosphatase was added. The chip was washed with HEPES buffer after incubation for 1 hour at room temperature. Finally, a certain concentration of the mixed substrate of disodium p-nitrophenyl phosphate (pNPP) and methylene blue (MB) was added into the chip. After reacting for 60 min at room temperature, a smartphone was used to take photographs and analyze the color signals of the solution in branch channels.

S9.3 Feasibility for detection of mycotoxins.

10 ng/mL OTA, AFB_1, FB_1 and the mixed sample solution were detected, respectively. Methylene blue concentration, the binding time of the probes and targets, Methylene blue concentration, the binding time of the probes and targets, pNPP concentration and substrate reaction time were 0.05 mg/mL, 60 min, 1 mg/mL and 60 min respectively.

S9.4 Optimization of experimental conditions.

To optimize the detection performance of three mycotoxins, the concentration of methylene blue in the mixed substrate was optimized.

In addition, some other influencing factors were also investigated, including the binding time between the probes and targets, the concentration of pNPP, and the reaction time of the mixed substrate. 0.1 ng/mL OTA was chosen as an example in the optimization procedure.

S9.5 Sensitivity for detection of mycotoxins.

Different concentrations of three mycotoxins (Table S3) were detected under optimized conditions. A smartphone was used to take photos and acquire the color signals of the solution in branch channels. In addition, 10 μL of the solution was also sucked out and diluted 10 times with 100 mM Tris-HCl buffer solution, and then analyzed with a microplate reader (Tecan M1000, Switzerland). Methylene blue concentration, the binding time of the probes and targets, pNPP concentration and substrate reaction time were 0.05 mg/mL, 90 min, 1.5 mg/mL and 60 min respectively

S9.6 Selective for detection of mycotoxins.

To investigate the selectivity for detection of the three mycotoxins, 10 ng/mL OTA, AFB_1, FB_1, GT, AFB_2, AFM_1, and a mixture of the above six mycotoxins were detected respectively in S-9 chip. Methylene blue concentration, the binding time of the probes and targets, pNPP concentration and substrate reaction time were 0.05 mg/mL, 90 min, 1.5 mg/mL and 60 min respectively

S9.7 Detection of mycotoxins in corn powder.

Weigh 0.2500 g negative corn powder and add 50 μL different concentrations of mycotoxins solution respectively (Table S4). These mixtures were dried overnight at room temperature to obtain spiked samples.

Add 1 mL 80% methanol to 0.2500 g corn powder spiked sample, which was extracted by vortex oscillation and sonic vibration for 3 minutes, respectively. Then, the mixture was filtered by a 0.45 μm needle filter. Finally, 200 μL filtrate was diluted by 800 μL HEPES buffer, and the contents of mycotoxins were measured.

The corn powder quality control products of OTA, AFB_1 and FB_1 were treated by similar methods. The contents of mycotoxins were measured, and the measured values were compared with the certified values.

S9.8 Comparison of our method for mycotoxins detection with the previous.

We compared our detection method with other methods for mycotoxins in terms of LOD, sample pretreatment and signal acquisition (Table S5).

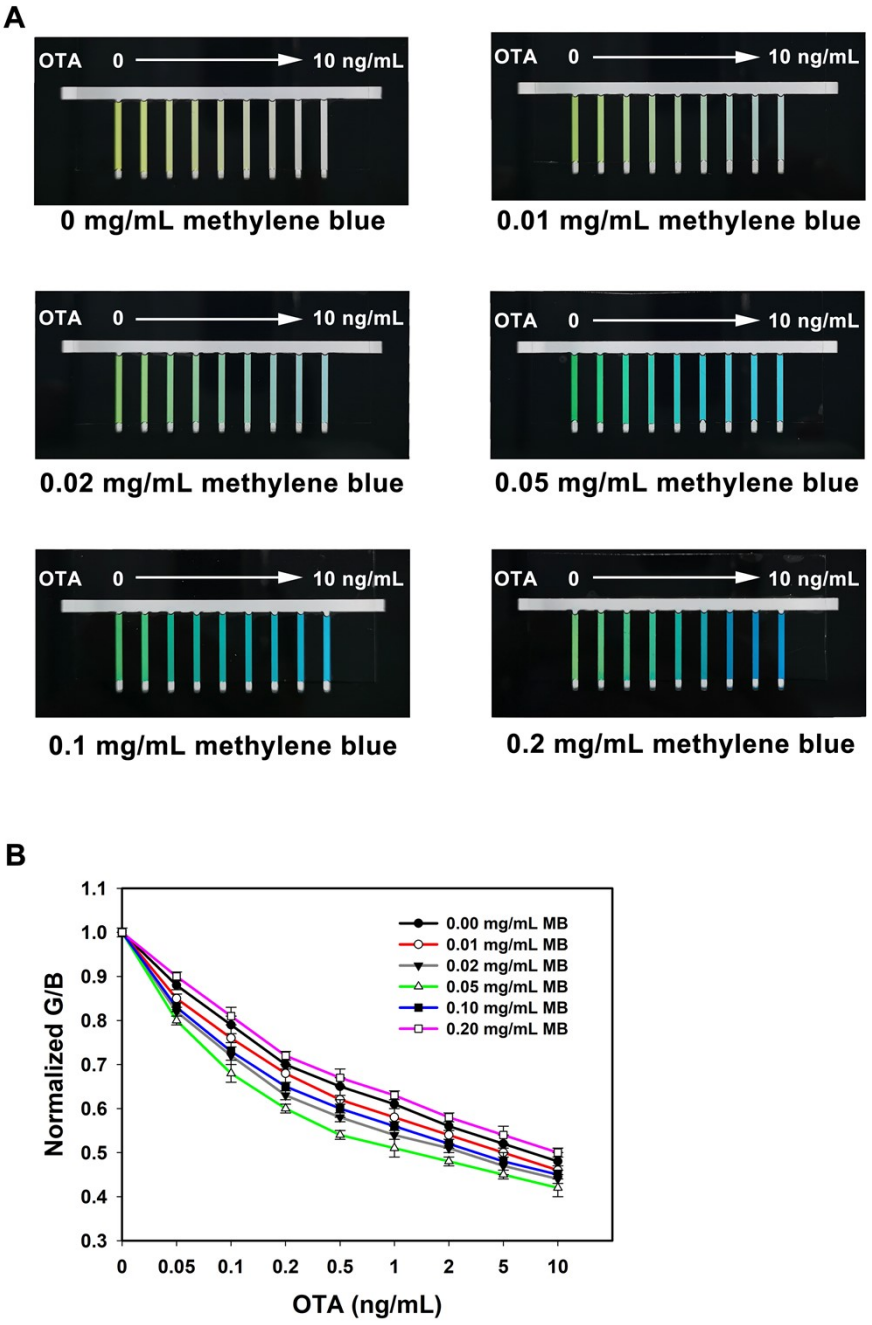


Fig. S7 Optimization of methylene blue concentration in mixed substrate. (A) The results in the S-9 chip. (B) The relationship between normalized G/B and different concentrations of OTA. Normalized G/B was obtained by dividing the G/B value for detection of different concentrations of OTA by the G/B value without OTA. The error bar represented the standard deviation of three parallel experiments.

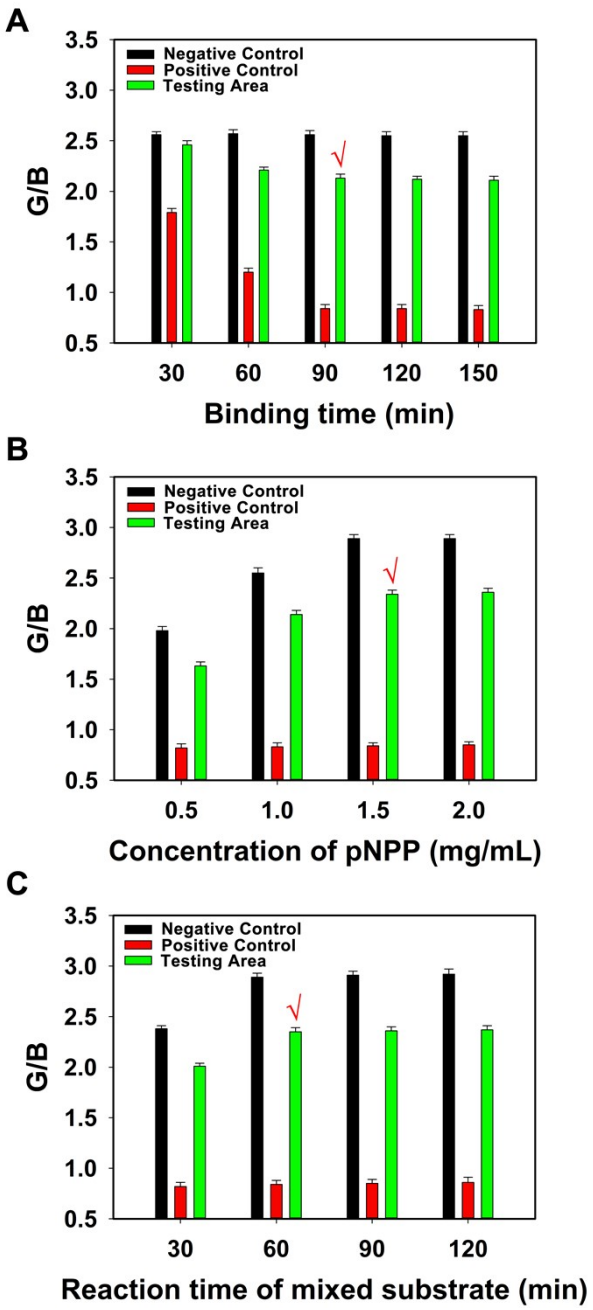


Fig. S8 The effects of the binding time between the probes and targets, the concentration of pNPP, and the reaction time of the mixed substrate on mycotoxins detection. Error bars represented the standard deviation of three parallel experiments.

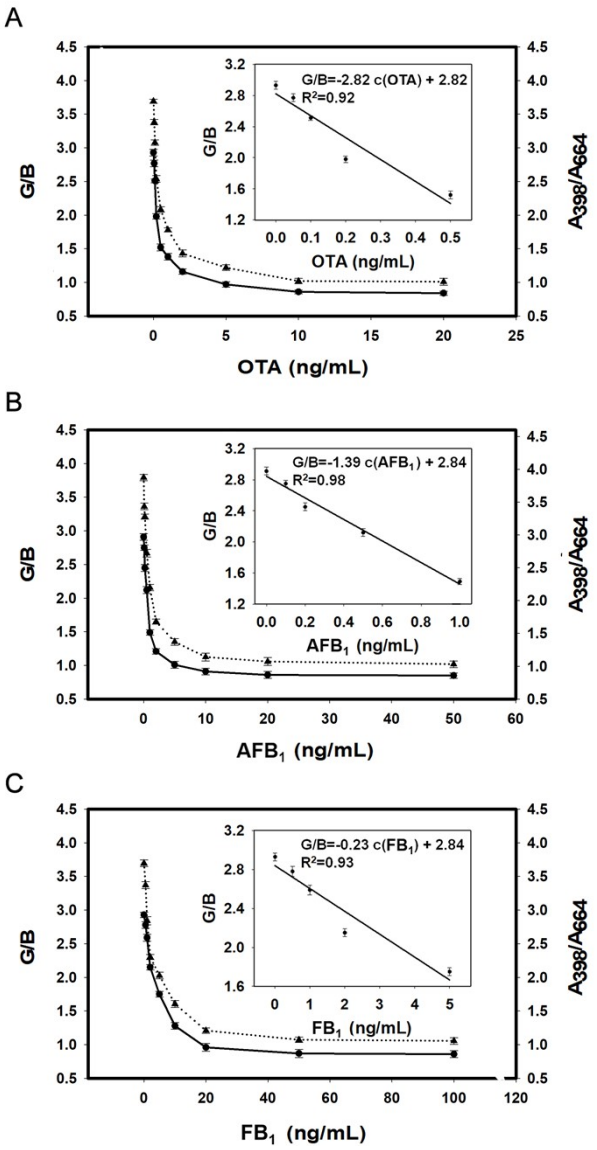


Fig. S9 Relationship between color signals and mycotoxin concentration. (A) OTA, (B) AFB1, and (C) FB1. The solid line (G/B) and dotted line (A398/A664) correspond to the measurement results of a smartphone and microplate reader, respectively. The insets show the linear fitting result of G/B and mycotoxin concentration. Methylene blue concentration, the binding time of the probes and targets, pNPP concentration and substrate reaction time were 0.05 mg mL^{-1}, 90 min, 1.5 mg mL^{-1} and 60 min respectively. The error bars represent the standard deviation of three parallel experiments.

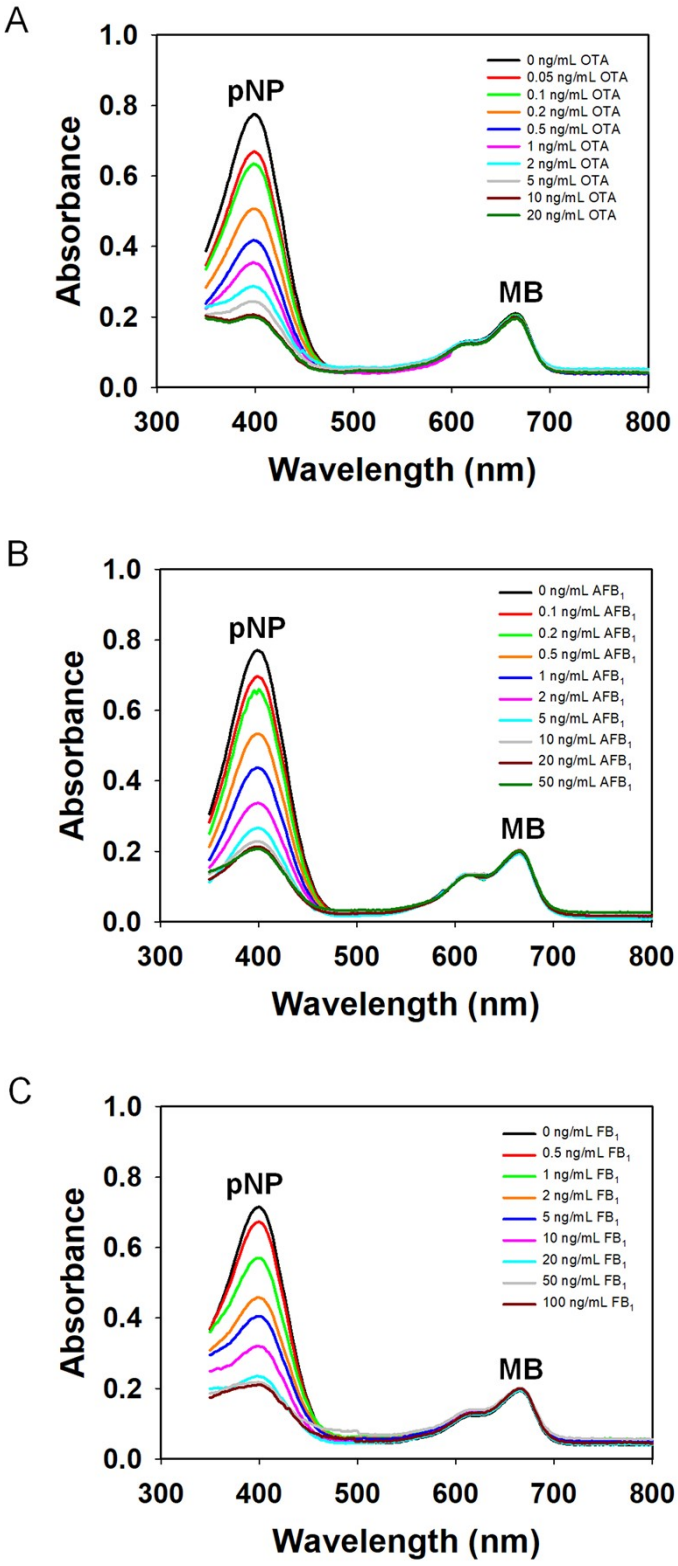


Fig. S10 The absorption spectra for detection of different concentrations of mycotoxins. (A), (B) and (C) were absorption spectra for detection of different concentrations of OTA, AFB_1, and FB_1, respectively.

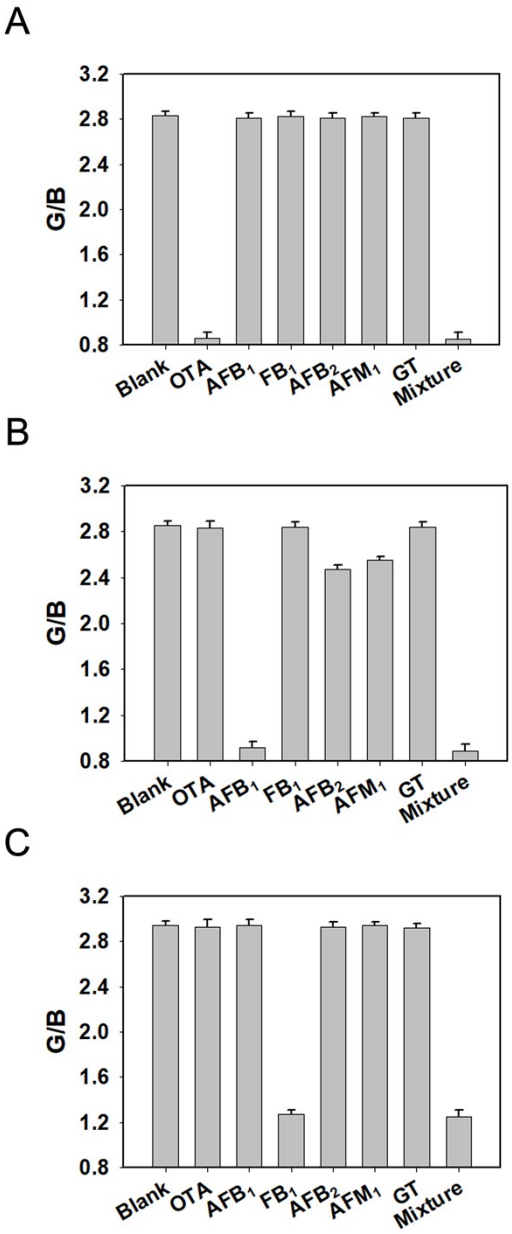


Fig. S11 Selectivity of detection of three mycotoxins. (A), (B) and (C) were the selectivity of branch channels modified with OTA, AFB_1 and FB_1 aptamer to different mycotoxins, respectively. Blank was the sample without mycotoxin. The mixture contained all six mycotoxins. The concentration of all mycotoxins was 10 ng/mL. The error bar represented the standard deviation of three parallel experiments. Methylene blue concentration, the binding time of the probes and targets, pNPP concentration and substrate reaction time were 0.05 mg/mL, 90 min, 1.5 mg/mL and 60 min respectively.

Table S3

Mycotoxins samples at different concentrations (Unit: ng/mL).

OTA	AFB_1	FB_1
0.00	0.00	0.00
0.05	0.10	0.50
0.10	0.20	1.00
0.20	0.50	2.00
0.50	1.00	5.00
1.00	2.00	10.00
2.00	5.00	20.00
5.00	10.00	50.00
10.00	20.00	100.00
20.00	50.00	---

Table S4

Preparation of the spiked samples.

Targets	Corn powder (g)	Mycotoxins solutions (μg/mL, 50 μL)	Spiked samples (μg/kg)
OTA	0.2500	0.025	5.0
	0.2500	0.10	20
	0.2500	0.25	50
AFB_1	0.2500	0.05	10
	0.2500	0.10	20
	0.2500	0.25	50
FB_1	0.2500	5.0	1000
	0.2500	10	2000
	0.2500	20	4000

Table S5

Comparison of the analytical performance of the assays for mycotoxins detection.

Analysis methods	Target	LOD (ng/mL)	Sample treatment	Signal acquisition	References
Fluorescence assay based on multicolor quantum dots strip	OTA AFB_1 FB_1	0.059 0.00165 1.58	Laboratory equipment	Fluorescent scanning reader	7
Fluorescence assay based on protein microarray on porous silicon	OTA AFB_1 FB_1	0.433 0.243 0.093	Laboratory equipment	Fluorescence microscope	8
Electrochemical assay based on aptasensor	OTA AFB_1	0.0133 0.0043	Laboratory equipment	Electrochemical workstation	9
Surface plasmon resonance assay	OTA AFB_1	1.27 0.59	Laboratory equipment	SPR instrument	10
Colorimetric assay based on pregnancy test strip	OTA AFB_1	0.05 0.02	Laboratory equipment	Smartphone	11
Colorimetric assay based on multicolor gold particle strip	OTA AFB_1 FB_1	0.10 0.06 3.27	Laboratory equipment	Digital scanner	12
Colorimetric assay based on multichannel microfluidic chip	OTA AFB_1 FB_1	0.02 0.06 0.13	Sonic toothbrush	Smartphone	This work

Table S6

Comparison of sonic toothbrush and vortex oscillator for mycotoxins extraction.

Targets	Spiked values (μg/kg)	Measured values (μg/kg, n=3)		Recovery (%, n=3)		P values
		sonic toothbrush	vortex oscillator	sonic toothbrush	vortex oscillator	
OTA	5.0	4.6 ± 0.3	4.8 ± 0.2	92 ± 6	96 ± 4	0.43
OTA	20	19 ± 1	18 ± 2	95 ± 5	90 ± 10	0.52
OTA	50	48 ± 1	49 ± 1	96 ± 2	98 ± 2	0.35
AFB_1	10	9.6 ± 0.4	10.2 ± 0.3	96 ± 4	102 ± 3	0.17
AFB_1	20	19 ± 2	18 ± 2	95 ± 10	90 ± 10	0.60
AFB_1	50	48 ± 3	49 ± 2	96 ± 6	98 ± 4	0.68
FB_1	1000	957 ± 17	969 ± 24	95.7 ± 1.7	96.9 ± 2.4	0.55
FB_1	2000	1957 ± 22	1968 ± 19	97.9 ± 1.1	98.4 ± 1.0	0.62
FB_1	4000	3808 ± 169	3787 ± 220	95.2 ± 4.2	94.7 ± 5.5	0.91

Table S7

Validation for detection of mycotoxins in corn powder.

Targets	Certified values* (n=30, μg/kg)	Measured values (n=3, μg/kg)	P values
OTA	18.0 ± 3.7	17.3 ± 1.9	0.80
AFB_1	9.3 ± 2.0	8.9 ± 1.1	0.79
FB_1	2300 ± 300	2317 ± 106	0.93

*The certified values were from the instructions of the corn powder quality control products of OTA, AFB_1, and FB_1. These corn powder quality control products were naturally contaminated samples purchased from Clover Technology Group Inc. (Manufacturer: Trilogy, USA). To reduce the influence of sampling heterogeneity, three parallel samples were used for analysis.

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