Electronic Supplementary Material (ESI) for Lab on a Chip.

Multiplex nanozymatic biosensing of *Salmonella* on a finger-actuated microfluidic chip

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Materials

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, Sigma), chloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$, Sigma), Sodium tetrachloropalladate(II) (Cl_4Na_2Pd, Sigma), trisodium citrate (Aladdin) and ascorbic acid (Aladdin) were used to synthesize the gold@platinum palladium (Au@PtPd) nanoparticles (1 mg/mL). Polyclonal antibodies (2.5 mg/mL, Fitzgerald) and monoclonal antibodies (2.5 mg/mL, Meridian) against *Salmonella typhimurium* were used to modify the magnetic nanobeads (180 nm, Allrun Nano) and Au@PtPd nanoparticles. Phosphate buffered (PB, 10 mM, pH 7.3, Solarbio) was used as buffer solution. Sodium hydroxide (NaOH, 1M, Sinopharm) was used to adjust the pH of PB. Bovine serum albumin (BSA, BIOFORXX) was dissolved in phosphate buffered saline (PBS, 10 mM, pH 7.4) for blocking. H_2O_2-TMB (Solarbio) was used as substrate for colorimetric reaction.

Detection of target bacteria in spiked pork meat

The practicability of this microfluidic biosensor was verified by detecting target bacteria in pork meats. Based on China's national standard for *Salmonella* detection, 25 g pork meats were first homogenized in 225 mL PBS to get the supernatant. Then, target bacteria, which were cultured for 22-24 h in LB Broth medium, were 10 times diluted by the supernatant with 1% BSA to get spiked pork samples at 1.6×10^3 - 1.6×10^6 CFU/mL. Finally, triplicate tests on each spiked sample were carried out using this biosensor and compared with the gold standard culture method.

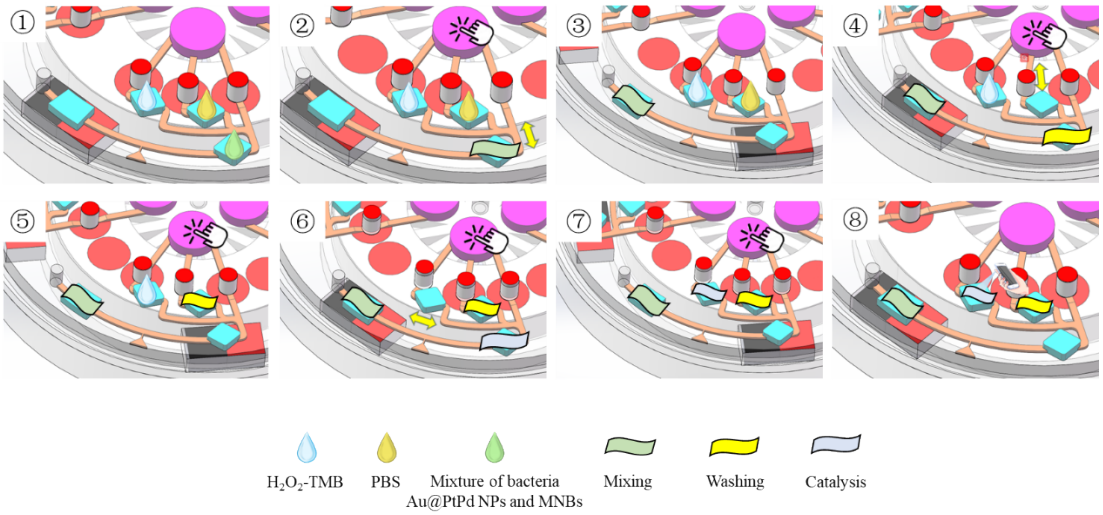


Fig. S1 The illustration of the procedure.

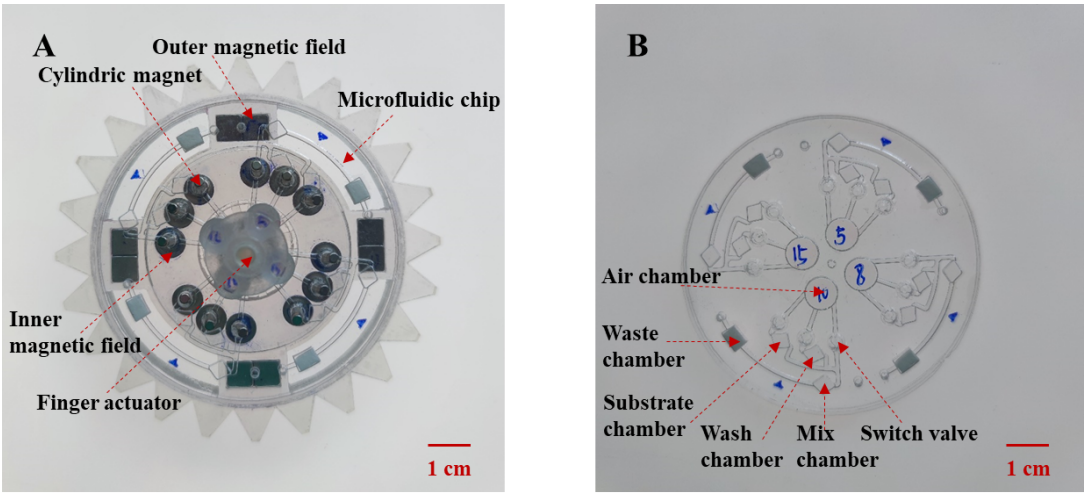


Fig. S2 The pictures of this biosensor. (A) This biosensor with a microfluidic chip, a finger actuator, two coaxial rotatable magnetic fields (the inner one the outer one) and 12 cylindric magnets. (B) The microfluidic chip with four parallel units (each unit included an air chamber, three cylindric holes as switch valves, a mix chamber, a wash chamber, a substrate chamber and a waste chamber).

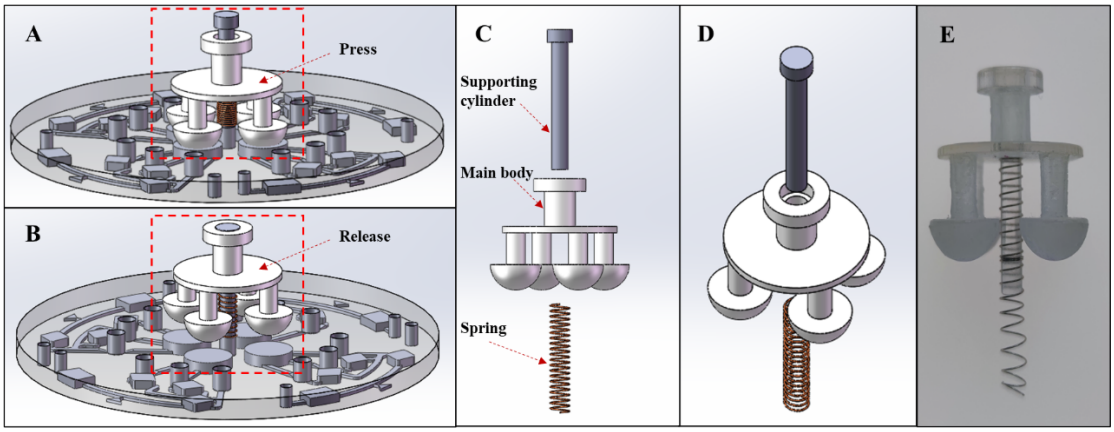


Fig. S3 The design of this finger actuator with a main body, a supporting cylinder and a spring. (A-B) The press and release of the finger actuator and the assembly of the microfluidic chip. (C-D) The multiple views of the finger actuator. (E) The picture of the finger actuator.

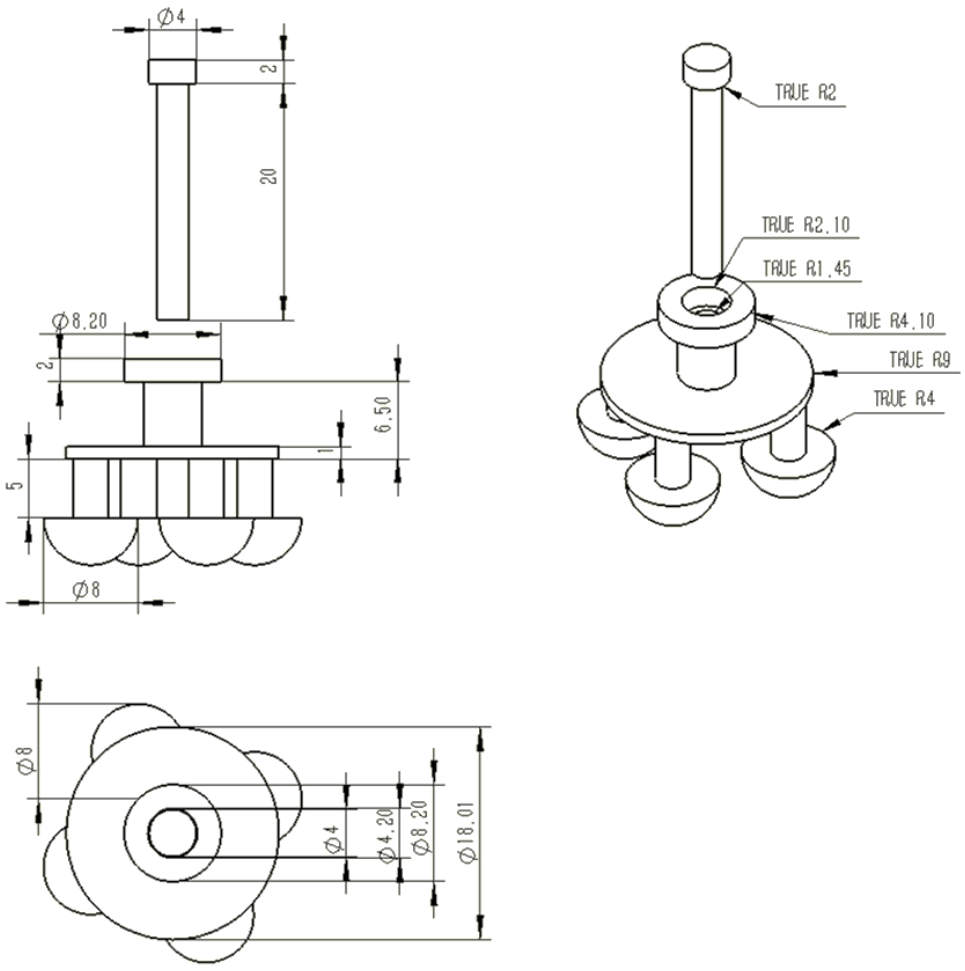


Fig. S4 The engineering drawing of the finger actuator (unit: mm).

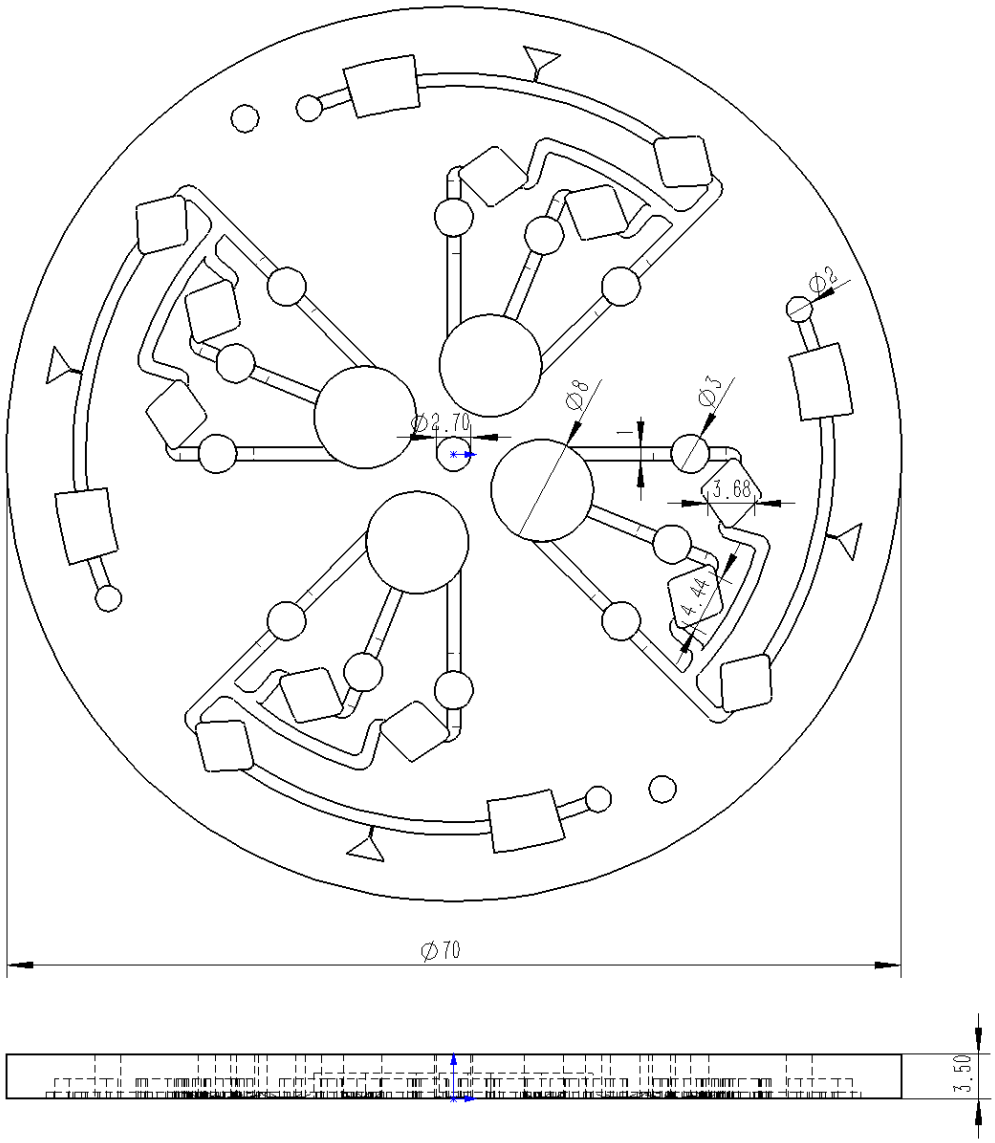


Fig. S5 The engineering drawing of the microfluidic chip (unit: mm).

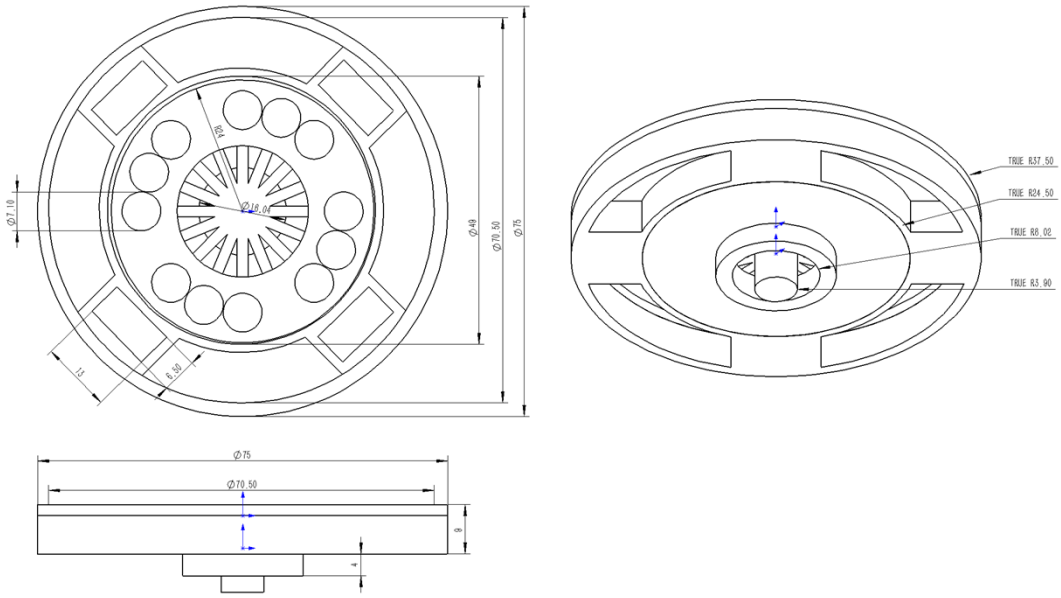


Fig. S6 The engineering drawing of the rotatable magnetic field (unit: mm).

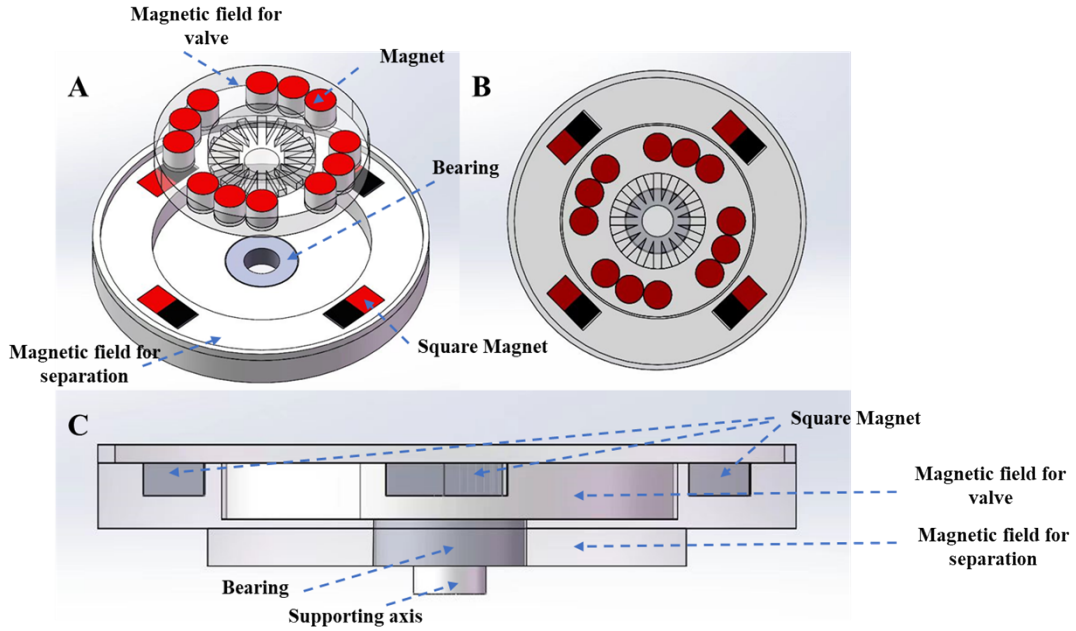


Fig. S7 The multi-view drawing of this coaxial rotatable magnetic field including the inner magnetic field and the outer magnetic field.

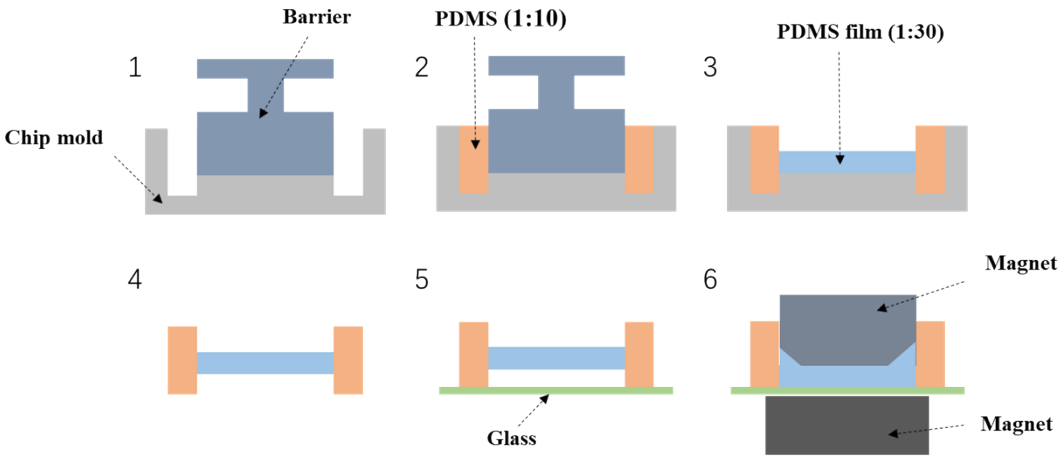


Fig. S8 The procedure for PDMS membrane construction in the valve.

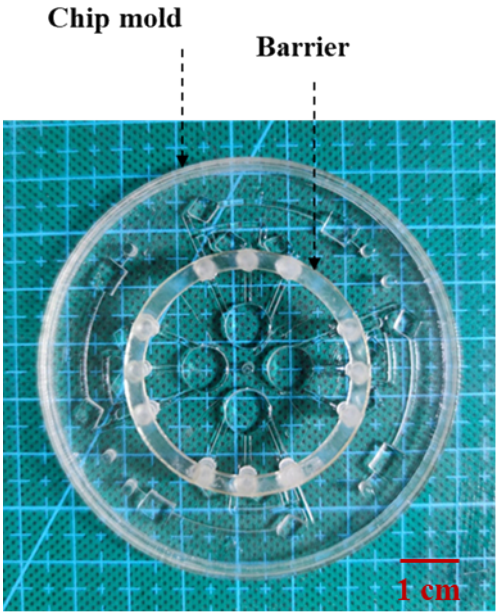


Fig. S9 The mold of this microfluidic chip with a barrier for forming 12 holes in the PDMS chips.

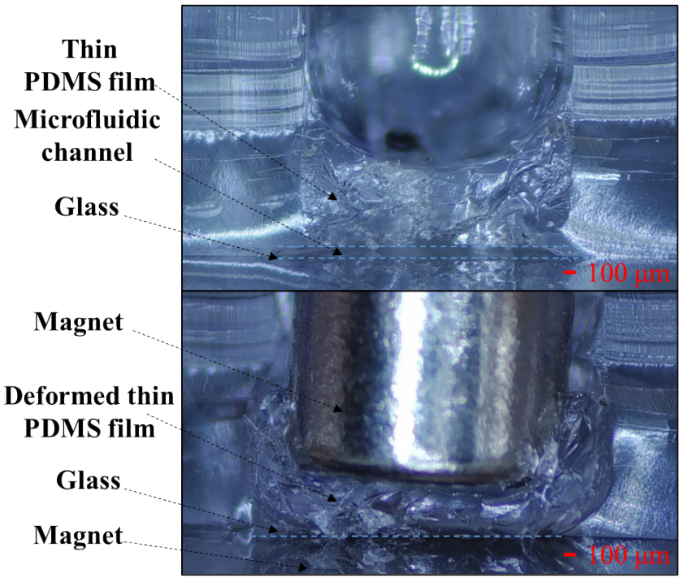


Fig. S10 The pictures of the valve when it was opened and closed.

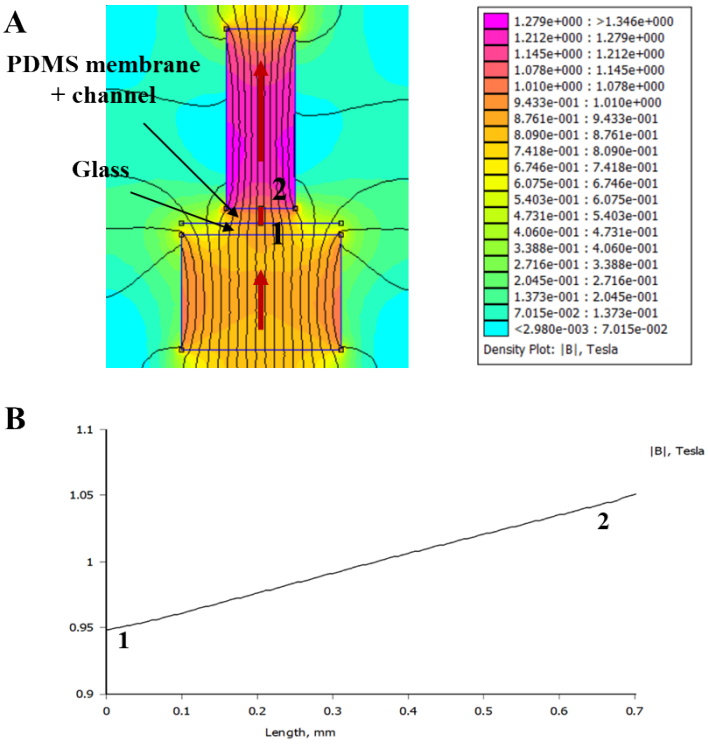
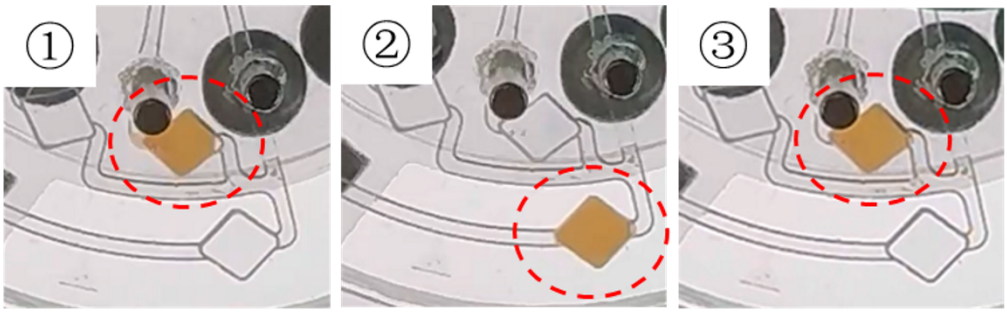


Fig. S11 The simulation of magnetic force between the magnet on the top of elastic thin membrane and the inner rotatable magnetic field when they were rotated into alignment.

A



B

	R	G	B
①	135.63	121.79	89.14
②	136.20	122.53	89.19
③	136.57	122.81	90.01

Fig. S12 Pictures for the movement of the MNBs. (A) 5 μg MNBs in 20 μL deionized water was first injected into the wash chamber and photographed (①), then moved to the mix chamber by pressing the air chamber and photographed (②), and finally returned back to the wash chamber after releasing the air chamber and photographed (③). (B) The RGB values of the MNBs in different steps.

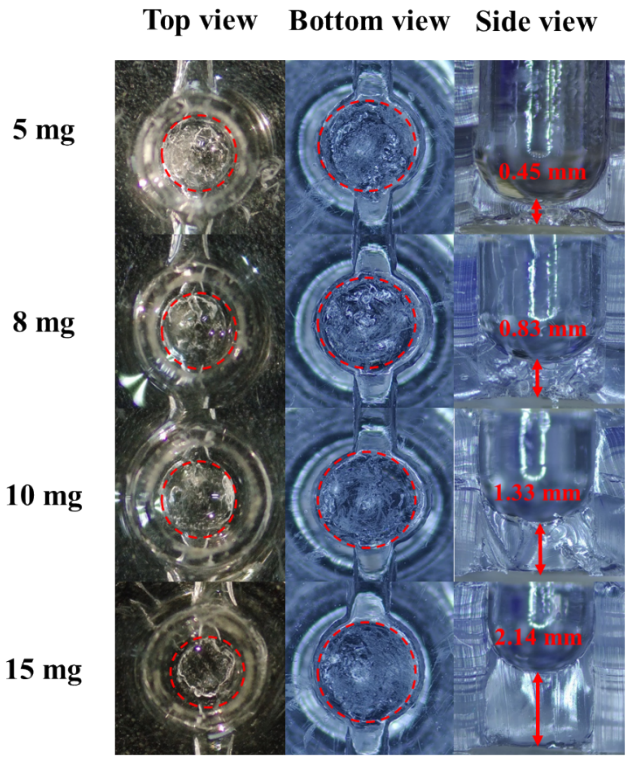


Fig. S13 The pictures of the membranes with different amounts (5 - 15 mg) of PDMS mixture.

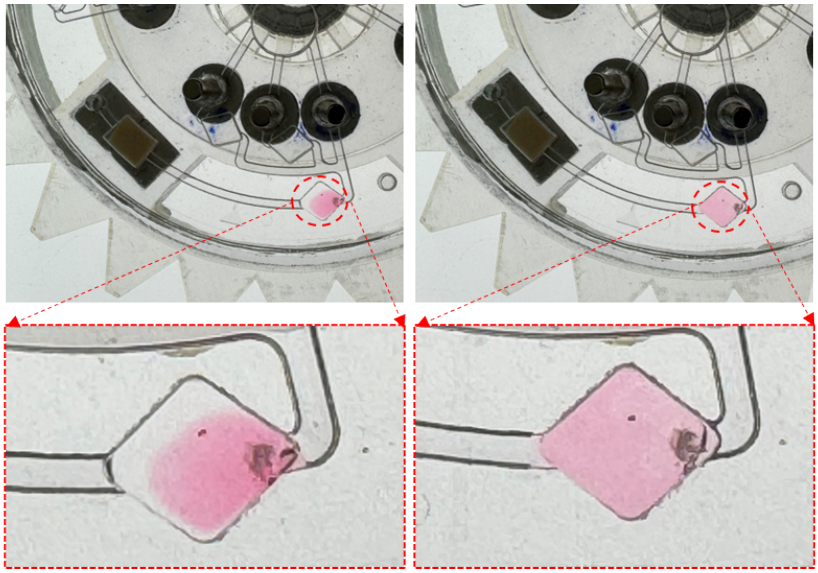


Fig. S14 Evaluation on the mixing effect of this finger actuated mixer using red dye and deionized water (The left was before mixing and the right was after 15 times of press-release actions).

Table S1 Detection of *Salmonella typhimurium* in spiked pork supernatant (N=3).

Culture (CFU/mL)	Biosensor (CFU/mL)	Recovery (%)	RSD (%)
1600	1390	86	3.56
16000	17434	108	3.04
160000	186956	116	4.19
1600000	1445931	90	3.18