Electronic Supplementary Material (ESI) for Analyst.
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Supplementary Information

A sample-to-answer DNA detection microfluidic system integrating sample pretreatment and smartphone-readable gradient plasmonic photothermal continuous-flow PCR

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§1. Synthesis of silica-coated gold nanorods:

The chemical reagents for gold nanorods preparation are all from China National Medicines Corporation Ltd. (Beijing), all of which are chemically pure. Laboratory-made ultrapure water (18MΩ*cm) was used for reagent preparation.

Gold nanorods were synthesized with seed mediated growth method and then modified with silica. Briefly, the seed solutions were prepared through mixing of hexadecyl trimethyl ammonium (CTAB) solution (10 mL, 0.1 M) and $HAuCl_4$ (0.085 mL, 0.028 M) with fresh $NaBH_4$ (0.07 mL, 0.1 M). For the growth of the gold nanorods, 0.3 mL seed solution was added to a mixture containing CTAB (12 mL, 0.1 M), sodium oleate (18 mL, 0.013 M), $HAuCl_4$ (0.5 mL, 0.028 M), HCl (1 mL, 0.1 M), $AgNO_3$ (0.32~0.37 mL, 0.01 M) and ascorbic acid (0.05 mL, 0.1 M). Following incubation at 30 °C for 24 h, the newly-produced AuNRs colloids were centrifuged at 10 000 rpm for 30 min, decanted, and resuspended in 30 mL of 1 mM CTAB to decrease free CTAB and sodium oleate. To enhance the stability of the gold nanorods at high temperature and the dispersion in PDMS, gold nanorods were then modified with silica using the Stober method. NaOH (0.1 M) was added dropwise to adjust the pH of AuNRs to 10.4~11.0. Next, 0.1 mL of tetraethylorthosilicate (TEOS) was added in AuNRs, shaken slowly for 1h, and then left 12 h for static growth at room temperature. A layer of silica was successfully coated onto the surface of the nanorods which were then centrifuged at 10 000 rpm for 20 min and removed supernatant. Wash twice with ethanol, and make up to 5 mL with ethanol to obtain gold nanorod-ethanol dispersion, and the relative mass of gold-nanorod is about 0.06%. It should be noted that the relative mass of gold in ethanol solution was determined by inductively coupled plasma source mass spectrometer (ICPS-MS), and the gold nanorods were stored at 4 °C before use.

§2. Digital PCR related operations:

Digital PCR-related operations used to assess and optimize DNA extraction efficiency were performed according to the manufacturer's instructions (Bio-Rad QX200, USA). The dPCR reaction mixture (20 μL) was prepared by mixing the following reagents: 10 μL QX200 ddPCR Supermix (Cat. No: 186-3010, Bio-Rad), 0.5 μL each of a pair of primers (forward and reverse), 0.25 μL of TaqMan probes, 1 μL purified template DNA (taken directly from elution chamber) and 8 μL ddH_2O. Then 20 μL dPCR mixture and 70 μL microdroplet generation oil (Cat. No: 1863004, Bio-Rad) are loaded in the the microdroplet generation chip to generate water-in-oil droplets with Drop Maker M1. Then the dPCR microdroplets was amplified with a T100 Thermal Cycler (Bio-Rad) as the following procedure: 95° C for 3 min, followed by 45 cycles of 94 ° C for 30 s and 60 ° C for 60 s. Subsequently, the amplified microdroplets were quantified by Chip Reader and analyzing software QuantaSoft 2.0. Each purified sample was tested by dPCR four times, and the average concentration was used to calculate the DNA extraction efficiency.

§3. Gel electrophoresis related operations：

5 μL of amplified products mixed with 1 μL of loading buffer (Cat. No: D1010, 6 ×, Solarbio) was resolved in 2% agarose gel (Cat. No: A8350, Solarbio) using 1 × TAE buffer (Cat. No: ST716, Beyotime) at 110 V (DYY-6C, Liuyi Biotechnology) for 70 ~ 80 min, with 10 μL green nucleic acid gel dye (Cat. No: G8140, Solarbio) staining. 5 μL DNA marker (Cat. No: DL 500 and Cat. No: DL1000 from Takara) was used to confirm product size. The gels were then imaged by a blue light gel imager (Sangon Biotech, Cat. No: 500312).

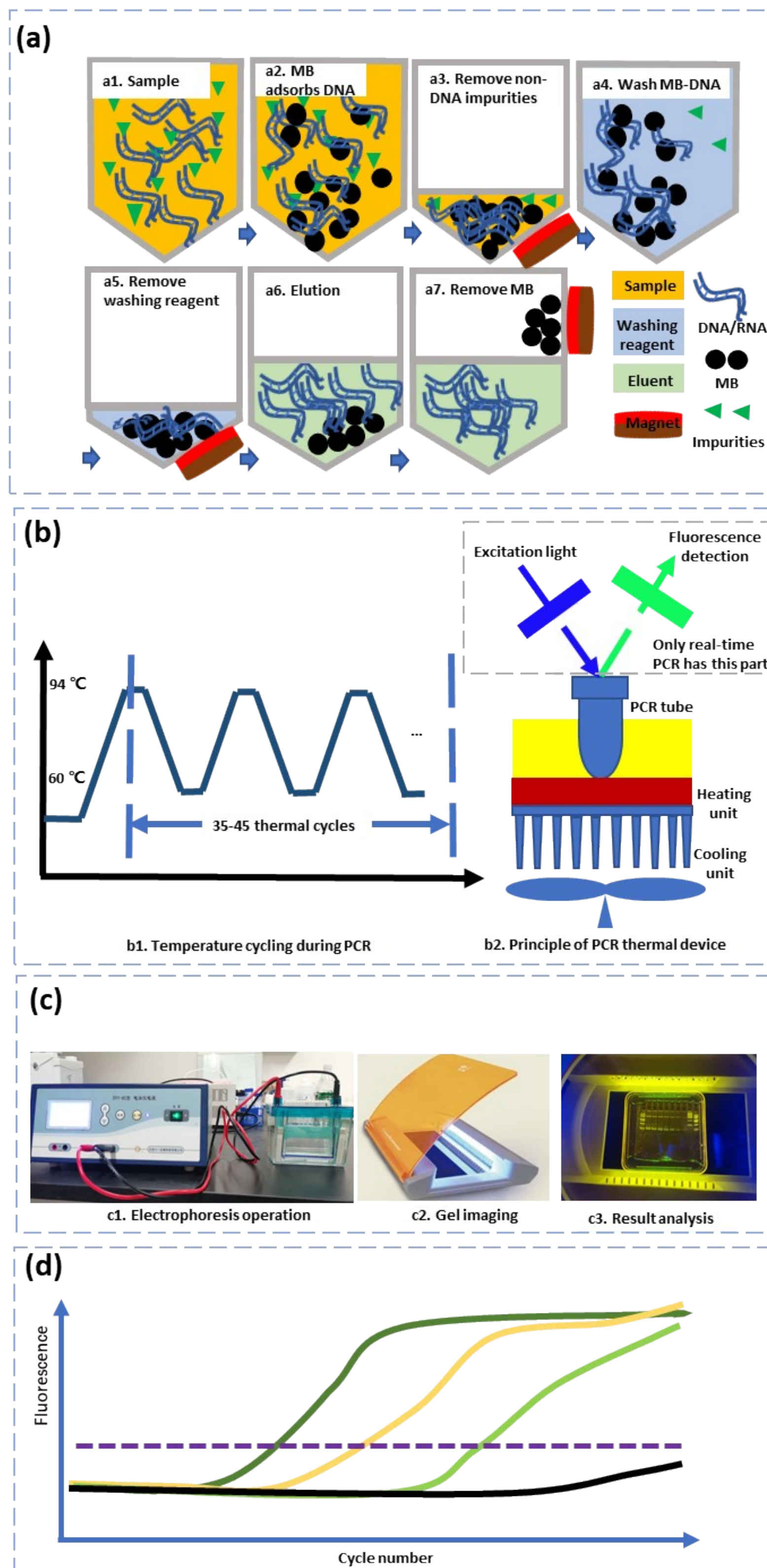
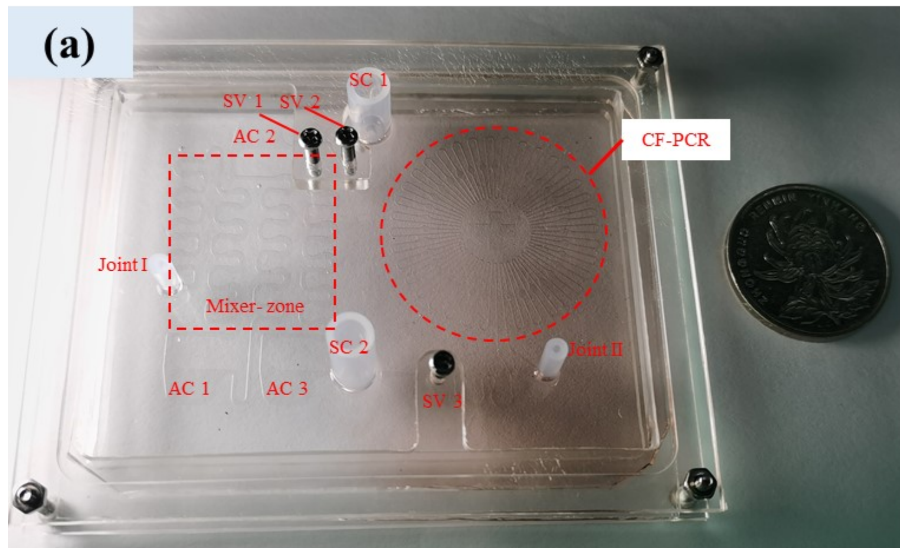
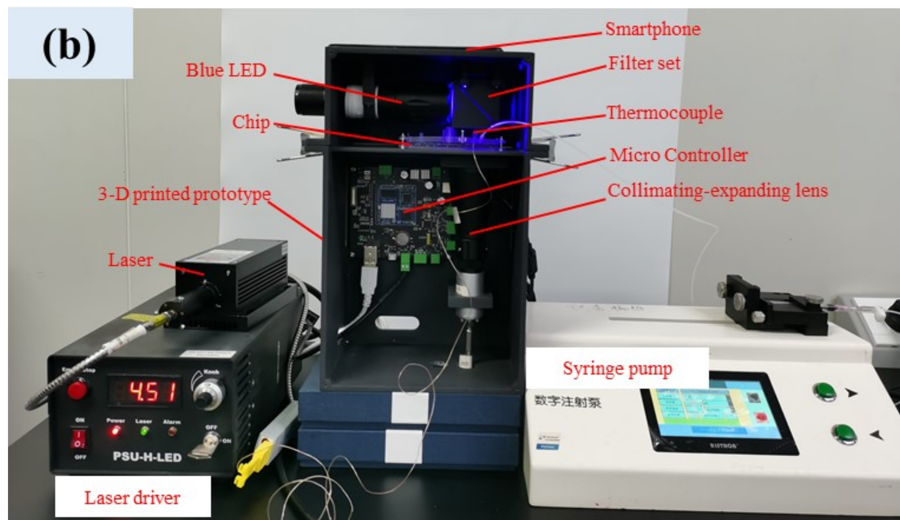


Fig. S1 Schematic diagram of the full process of nucleic acid detection by PCR from sample to answer：(a) sample pretreatment based on MB operation, (b) schematic diagram of thermal cycle and equipment for PCR, (c) DNA content characterization by electrophoresis, and (d) DNA content characterization by real-time fluorescence.





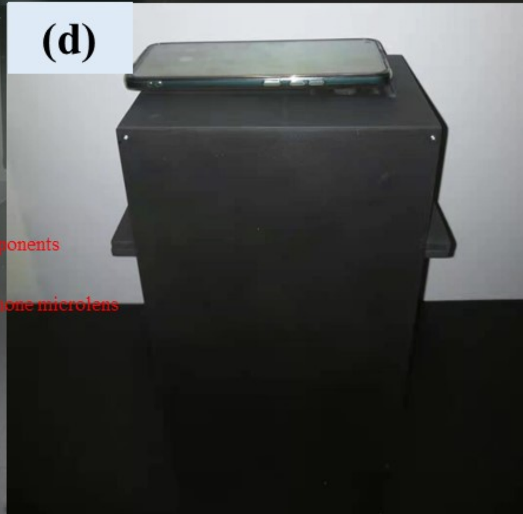
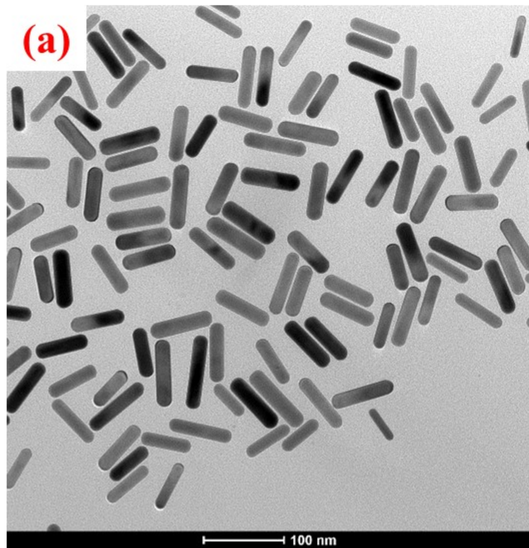


Fig. S2 Appearance images of (a) integrated microfluidic chip, (b) prototype system, (c) fluorescence detection device components， and (d) shading design of fluorescence detection.



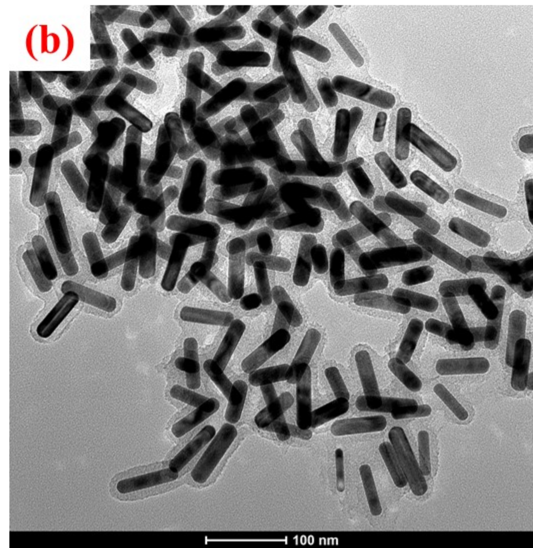
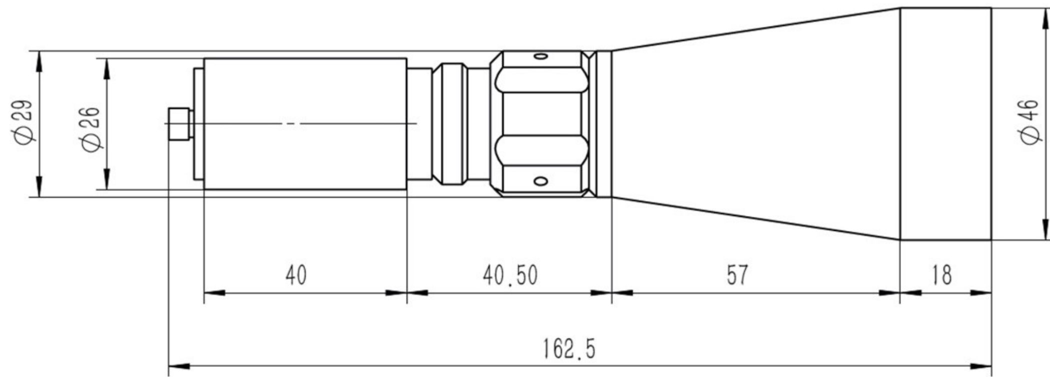
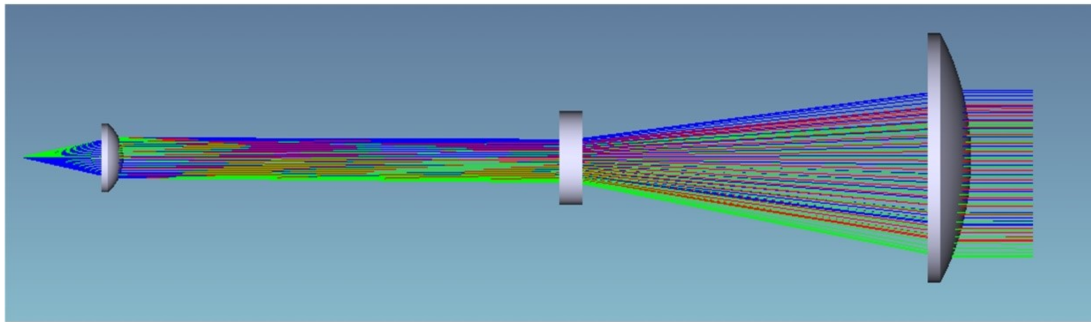


Fig. S3 Transmission Electron Microscope (TEM) images of (a) AuNRs, and (b) the silica coated AuNPs.



(a)



(b)

Fig. S4 (a) Appearance dimensions of the homemade collimating-expanding lens. (b) Schematic diagram of the lens arrangement and the trace of the beam collimation and expansion in Zemax.

Table S1 Detailed design parameters of the homemade collimating-expanding lens.

Surface：type		Radius	Thickness	Glass	Semi-Diameter	Conic	Par 0	Par 1	Par 2
Object	Standard	Infinity	12.41		0.2	0	/	/	/
1	Standard	Infinity	3.500	H-K9	5.5	0	/	/	/
2	Even Asphere	-7.363	70.00		5.5	0	0	$5.04*10^{-4}$	$-1.73*10^{-5}$
3	Standard	-488.528	2.00	H-K9L	7.5	0	/	/	/
4	Standard	11.919	57.00		6.0	0	/	/	/
5	Standard	Infinity	7.00	H-K9L	20.0	0	/	/	/
6	Standard	-43.160	10.00		20.0	0	/	/	/
Image	Standard	Infinity	/		18	0	/	/	/

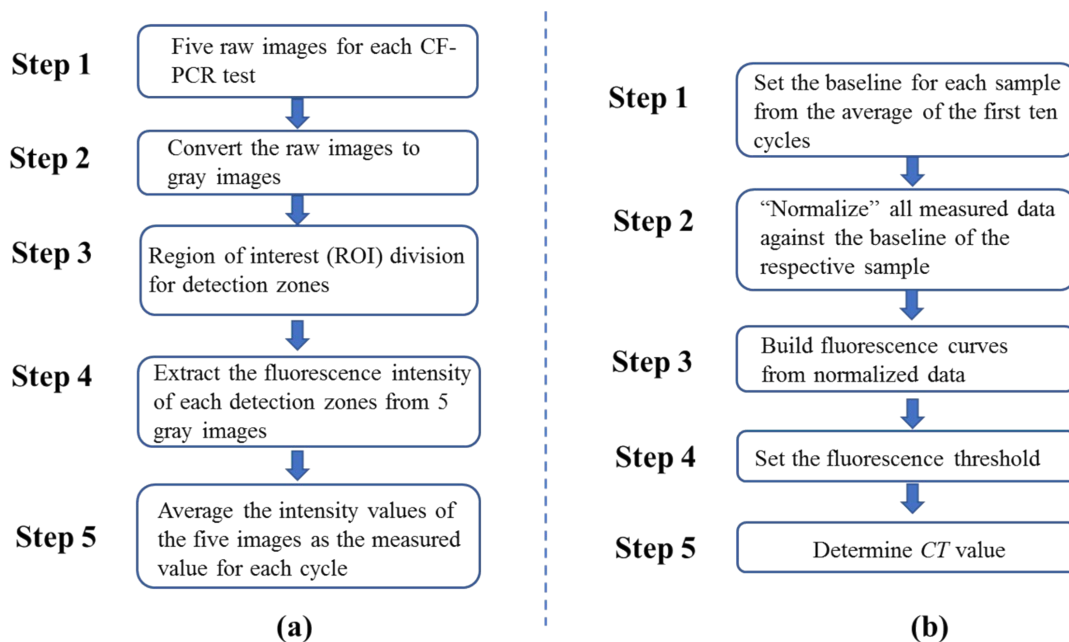


Fig. S5 Data processing pipeline for CF-PCR. (a) Process flow chart for image processing for real time CF-PCR, (b) Flow chart of amplification curve construction.

During fluorescence detection, five images were collected sequentially for each sample at a 2-minutes interval, and then the average gray value of the detection zones of the five images is used as the measured fluorescence intensity to reduce imaging errors caused by changes in excitation light, fluctuation in liquid flow, and air bubbles. When constructing the amplification curve, considering the interference of reagent loading error, microflow channel manufacturing error, background fluorescence and phone camera background noise, the measured fluorescence intensity of the initial 10 cycles should be used as the baseline to "normalize" the measured data. It should be noted that the normalization here is to artificially set the initial fluorescence of different samples to a uniform level, so that the amplification curves of batch experiments have a uniform fluorescence threshold.

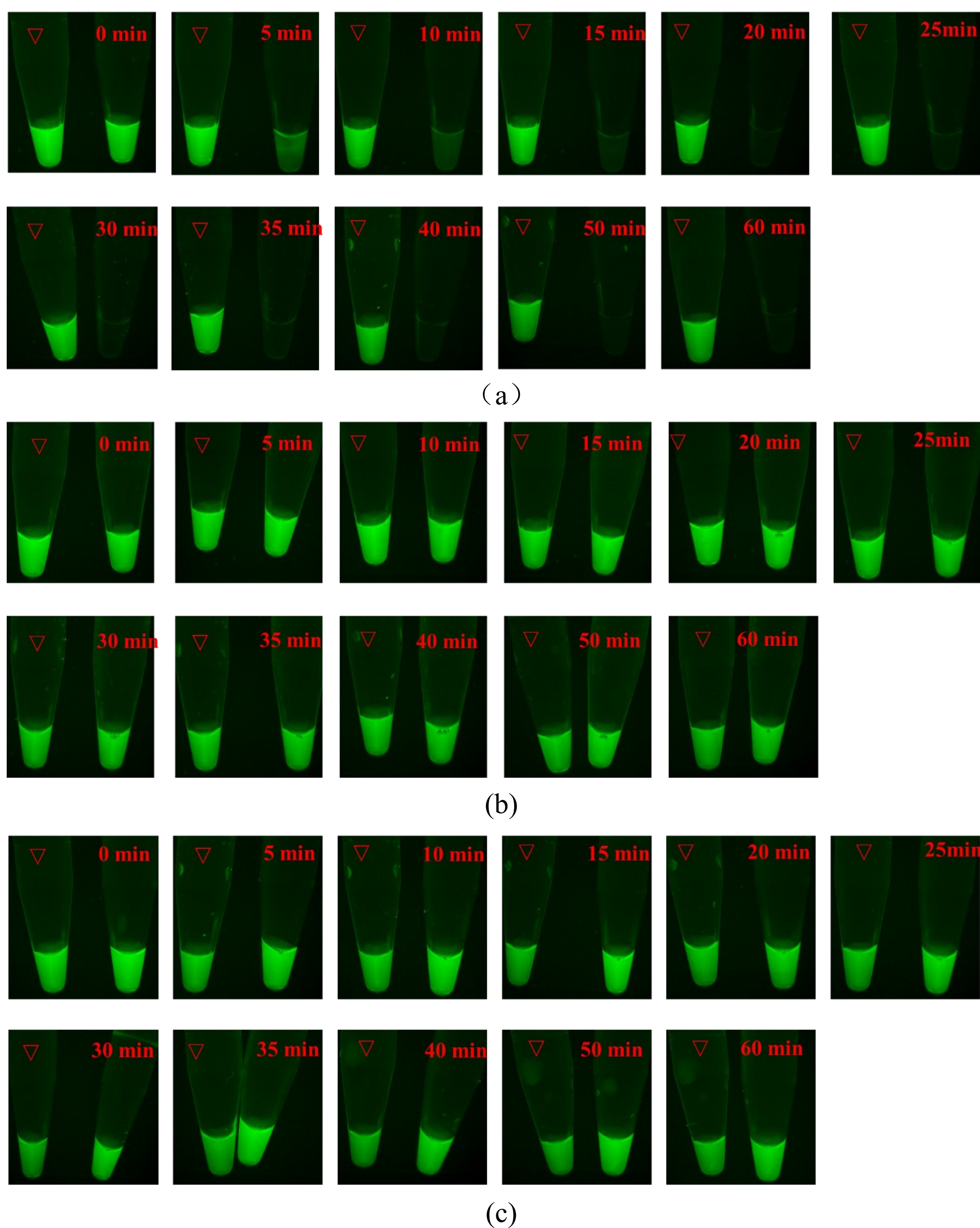


Fig. S6 Fluorescence images of PCR products exposed to light at (a) 450~480 nm, (b)808 nm and (c) 820~850 nm, respectively.

In Fig. S6, each left tube with a triangle symbol (▽) is a reference tube stored in the dark, each right tube is exposed PCR product, and the corresponding exposure time is also marked on each right tube. The fluorescence signal of the PCR product irradiated by 450~480 nm light showed a significant decrease. However, the fluorescence signal of PCR products remained almost unchanged at 808 and 820~850 nm irradiation even for one hour. This indicates that 808 nm laser can realize the photothermal amplification, and avoid the photobleaching of SYBR green dyes.

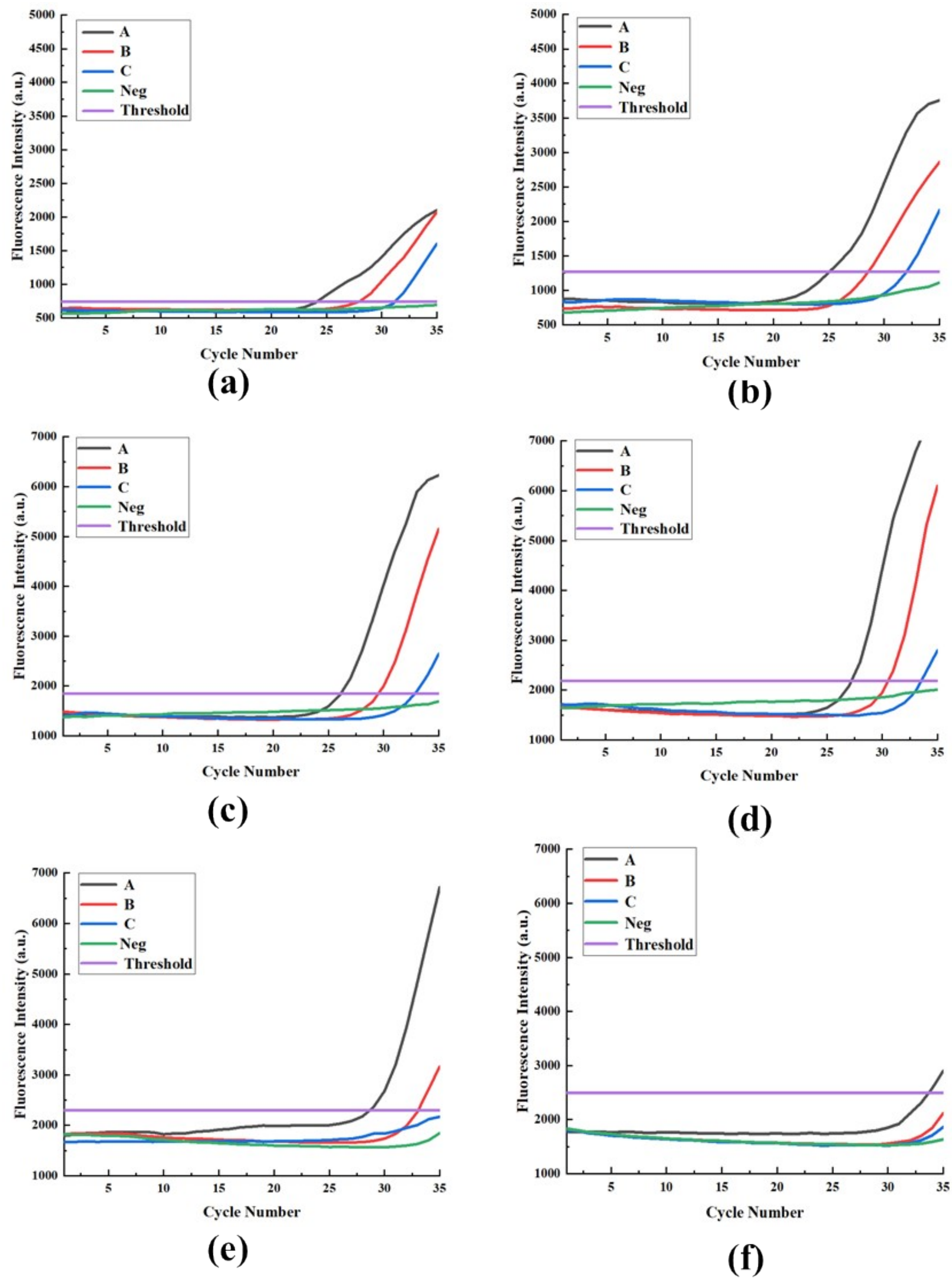


Fig. S7 Real-time fluorescence curves of PCR mixtures labeled with different SYBR green (SG) dyes. From (a) to (f), the relative content of SG dyes gradually increased from 0.5 × to 4 ×.

Table S2 Summary of the results of real-time PCR experiments in Fig. S7.

SG Content	$F_{threshold}$	CT_A	CT_B	CT_C	CT_{Neg}	$F_{initial}$	F_{end}
(a) 0.5 ×	743	24.4	27.8	31.3	/	603	2153
(b) 1 ×	1267	25.0	28.4	32.0	/	844	3755
(c) 2 ×	1872	25.7	29.1	32.8	/	1435	6230
(d) 2.5 ×	2213	26.7	30.4	33.8	/	1684	7299
(e) 3 ×	2337	28.9	33.0	/	/	1804	6722
(f) 4 ×	2509	33.4	/	/	/	1859	2903

In Fig. S7 and Table S2, all real-time PCR experiments were performed based on a commercial PCR instrument (Gentier 96E, Xi`an Tianlong, China), and the initial HBV-DNA concentrations corresponding to A, B and C (20 µL mixture) were 2000, 200 and 20 copies/µL, respectively. The SG dye contents of PCR reagents in sub-figures of (a), (b), (c), (d), (e), and (f) are 0.5 ×, 1 ×, 2 ×, 2.5 ×, 3 ×, and 4 ×, respectively. The fluorescence threshold ($F_{threshold}$) and cycle threshold (CT) of each experiment are provided by the algorithms of Gentier 96E instrument, and the average fluorescence at the initial stage ($F_{initial}$) and end point (F_{end}) come from the data of each sample A, respectively.

As shown in Fig. S7 and Table S2, the PCR reaction failed when the SG dye was as high as 3 × to 4 ×, suggesting that SG dye concentrations above 3 × can inhibit the PCR reaction. When the SG dye is lower than 2.5 ×, the nucleic acid samples were successfully amplified, indicating that the SG dye within 2.5 × is compatible with this PCR mixture system. With the increase of SG dye (0.5 × ~ 2.5 ×), the initial fluorescence ($F_{initial}$) and end-point fluorescence (F_{end}) will increase, which is beneficial to the smartphone-based imaging. Therefore, we adopted 2 × SG dye to maintain the success rate of PCR and improve the fluorescence signal in our CF-PCR chip, and finally overcome the obstacle of insufficient light transmittance of AuNRS-PDMS material.

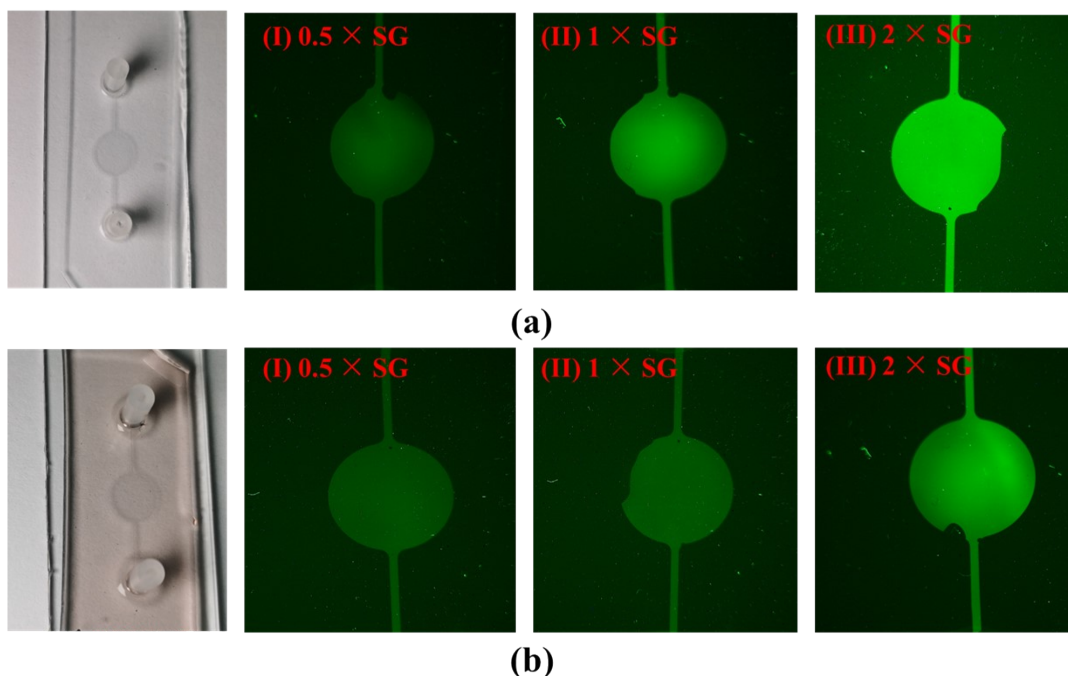


Fig. S8 Fluorescence images of PCR products (0.5 × to 2 × SG dye-labeled) loaded in (a) PDMS and (b) AuNRs-PDMS test chips.

In Fig. S8, the PCR products was dispensed from an amplified PCR tube, and the SG dye was added separately after amplification to ensure the same relative content of HBV-DNA fragments in each test chip. From those images, it can be found that the introduction of AuNRs reduces the fluorescence signal. When the SG dye was increased from 0.5 × recommended by conventional experiments to 2 ×, the AuNRs-PDMS chip presented a fairly clear fluorescence image. Therefore, although the absorption peak of AuNRs around 518 nm affects the transparency of AuNRs-PDMS to visible light, increasing the concentration of the SG dye in PCR mixture can facilitate the realization of fluorescence detection.

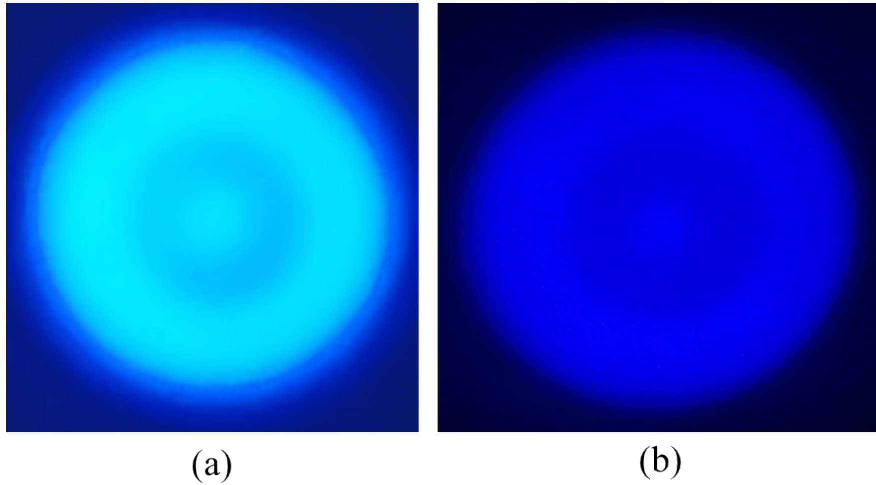


Fig. S9 Edge effects or inhomogeneities in common light sources.

In Figure S9, we show the spot images of two LED light sources(a: M470L3-C2 Thorlabs, b：E17, Alongefire). Although the spot images are not strictly instrumental representations, it can be intuitively seen from these images that the spatial distribution of optical power is not uniform, showing significant radial variation, while at the same circumference, the intensity is relatively uniform. Therefore, it is conceivable that the circular layout of the detection points with equal diameters can make the excitation light powers nearly equal, thereby ignoring the excitation light inhomogeneity for fluorescence imaging.

Table S3 Cost constraints during system and prototype development.

Parts name	Type specification and manufacturer	Cost of parts (USD)
3D-Printed prototype	Dimensions: 150 mm (long) *125mm (wide) * 285mm (High). Substrate: Polycarbonate (Polyjet), 380g. Manufacturer: Xi'an Zanglei 3-D Technology Co., Ltd.	210
Smartphone	Type: Honor 30 BMH-AN10, Huawei Technologies Co., Ltd. Specification: 40 million pixels (F/1.8).	390
Macrolens for phone	Type: Beetle 1678, Fuyan Optical Co., Ltd., Wuhan, China. 40~75 mm focal length, 5 × magnification.	45
NIR Laser	Type: LR-MFJ-808/5000mW, Lei Rui Optoelectronics Co., Ltd. Changchun, China. 808nm, 0~5W, Adjustable output with TTL control, Fiber output.	525
Laser collimating -expanding lens	Customized by Liguang Optoelectronics Entrance pupil: Φ10 mm, Magnification: 3.6	80
Filter set	Excitation filter: FF01-485/28, φ25 mm. Dichroic mirror: FF497-Di01, 25 mm×36 mm. Fluorescence filter: FF01-525/39, φ25 mm. Both from Witlan Industry Co., Ltd, Shanghai, China.	430
Bule LED flashlight	Type: Alongefire, E17, China. Specification: 450~490 nm, 0.47 W.	13
Temperature controller	Customized by Youfan Electronics, Dongguan, China. Measurement range: -20~125 °C. Accuracy: ±0.2℃. Output: TTL, 3000k Hz, 5V.	115
Syringe pump	Type: LSP01-1A, Dichuang Precision Electronics Co., Ltd., Shenyang, China. Flow rate range: 0.1~1000μL/min, Accuracy: <5%.	620
Total cost	2428	

Table S4 Comparative analysis with commercial instruments.

Required instrument configuration*	① Nucleic acid extraction instrument ② Real-time PCR instrument	① Nucleic acid extraction instrument ③ Thermal cycler ④ Electrophoresis special regulated power supply ⑤ Blue light gel imager	This study**
Representative instrument models	① NP968-C (Tianlong Biotechnology, Xi`an, China) ② Gentier 96R (Tianlong Biotechnology, Xi`an, China)	① NP968-C (Tianlong Biotechnology, Xi`an, China) ③ Gentier 32R (Tianlong Biotechnology, Xi`an, China) ④ DYY-6C (Liuyi Biotechnology, Beijing, China) ⑤ WD-9403X (Liuyi Biotechnology, Beijing, China)	/
Equipment cost	① 8900 +②18500 =27400 USD	① 8900+③ 6800 +④ 880 +⑤ 450=17030 USD	2428 USD
Footprint and weight	① 400 mm*420 mm*470 mm, 37 kg ② 295 mm*550 mm*495 mm, 35 kg	① 400 mm*420 mm*470 mm, 37 kg ③ 260 mm*400 mm*260 mm, 11 kg ④ 235 mm*295 mm*95 mm, 2.5 kg ⑤ 190 mm* 205 mm*15 mm, 1kg	3-D printed protype: 150 mm*125 mm*285 mm, 0.38 kg NIR laser: 130 mm*160 mm *85 mm, 1.3 kg Pump：240 mm* 175mm* 90mm, 1.9 kg
Sample pretreatment	Required sample volume: 100~1000μL Extraction time：20~40 min	Required sample volume: 100~1000μL Extraction time：20~40 min	Required sample volume: 100μL Extraction time：14 min
PCR amplification	Reaction volume: 10~100 μL Amplification time: 60~80 min	Reaction volume: 10~100 μL Amplification time: 60~80 min	Reaction volume: 40 μL Amplification time: 23 min
Quantification performance	Analysis method: real-time fluorescence Post-PCR operation time: instantly Minimum detection limit: 100 copies Dynamic range: 7 orders of magnitude	Analysis method: Electrophoresis Post-PCR operation time: 80 min for electrophoresis + 5 min for staining + 1 min for gel imaging=86 min Minimum detection limit: 100 copies Dynamic range: qualitative output	Analysis method: real-time fluorescence Post-PCR operation time:<5 min Minimum detection limit: 414 copies/reaction Dynamic range: 6 orders of magnitude
Remark	*: Those commercial instrument configurations are common product models with high cost performance in the Chinese market, and the price here is also the lowest price published by the network. **: For specific models and costs, see Table. S3.		

Table S5 Comparative analysis with recently multifunctional microfluidic scheme.

References	Scheme principle and auxiliary equipment	Function and performance
34	Dual-axis centrifugal microfluidics for DNA extraction Liquid moving between two thermal chambers for fast PCR CCD-based synchronous fluorescence device Dual-axis centrifugal operating platform Machined multilayer PMMA chip	Raw sample: HBV-DNA blood, 500 µL DNA extraction time: 15min DNA recovery rate: equivalent to commercial instruments Amplification time: 33 min Minimum detection limit: 50 copies, quantitative result
21	Gravity-driven cell enrichment and photothermal lysis LED-driven plasmonic photothermal device Reaction tube embedded with porous gold nanofilm Post-PCR endpoint fluorescence analysis	Raw sample: E. coli bacterial, 1000 µL DNA extraction time: 3 min Amplification time: 13 min Minimum detection limit: 10^3 CFU/mL Post-PCR analysis time: 10 min, qualitative result
9	Continuous-flow PCR device with two heating sources Self-made injection device PC injection molded CF-PCR chip High voltage power supply for electrophoresis	No sample pretreatment Amplification time: 151s Electrophoresis detection time: 223s Minimum detection limit: 125 CFU/µL, qualitative result
36	Nucleic acid extraction co-driven by MB operation, Closed-loop convective PCR reactor CMOS-based synchronous fluorescence detection Pump, vibration motor, and laser	Raw sample: Salmonella, 100 µL DNA extraction time: 3 min DNA recovery rate: 27% (Derived by *CT* value difference) Amplification time: 25 min Dynamic linear range:10^6~10^1 copies/µL, quantitative result
31	Manual nucleic acid extraction based on immiscible phase reagents Integrated PDMS chip for DNA extraction and digital LAMP Smartphone-based endpoint fluorescence detection Injection pump and benchtop heater	Raw sample: HBV-DNA plasma, 250 µL DNA extraction time: 15 min DNA recovery rate: 75% (Measured by digital PCR) Amplification time: 45 min Dynamic linear range:10^4~10^1 copies/mL, absolute quantitative result
53	Manual DNA extraction based on a pipette-driven capillary array comb LAMP amplification Endpoint fluorescence detection Benchtop heater, mixer and centrifuge	Raw sample: Bacteriophage λ-DNA, 50 µL DNA extraction time: 20 min Amplification time: 65 min Minimum detection limit: 500 copies, qualitative result
54	Manual DNA extraction based on electrostatic adsorption PMMA machined CF-PCR chip Nikon Eclipse TE 2000-U microscope for endpoint fluorescence detection Dual zone heater	Raw sample: E. coli O157:H7 lysis, 5 µL DNA extraction time: 21 min Amplification time: 35 min Minimum detection limit: 2500 CFU, qualitative result
This study	Pump-driven continuous injection of immiscible reagents for nucleic acid extraction GPPT-driven CF-PCR Smartphone-based real-time fluorescence detection PDMS microfluidic chip, laser, pump	Raw sample: HBV-DNA plasma, 100 µL DNA extraction time: 14 min DNA recovery rate: 69% (Measured by digital PCR) Amplification time: 23 min Dynamic linear range:10^6~10^1 copies/µL, quantitative result