

Supplemental Materials

Supplemental Material S1. Python code for plasmonic dPCR sensor system

The Python code to control the plasmonic dPCR sensor system can be downloaded from Github under the following link: <https://github.com/cDNAg1/dPCR-Device.git>. All variables required by the code as well as experimental data acquired by the device during a dPCR run is saved in an SQL database (Cyclingdata.db) that also can be found on the Github. For thermal cycling, the device measures the resistance of the gold wire structure by reading the value of the ADC. The ADC readout is then converted into a temperature using the parameters entered into the database. An error is calculated from the difference to the set temperature and the error used to calculate a response from a PID controller. Afterwards, the PID response is converted into a pulse width modulation, which is passed to the LEDs. During cycling, all measured values (ADC value, voltages, measured temperatures) and output values (PID value, PWM) are saved to a database for later extraction by the user, if so desired.

Supplemental Material S2. Theoretical determination of the detection range of the system

The average number of target molecules per well can be determined under the assumption of a Poisson distribution of target molecules into the microwells. The average number of target molecules (λ) can in this case be calculated from the probability of having no target molecules in a given well:

$$P(x = 0) = e^{-\lambda}$$

The probability of an empty well can be determined experimentally. Further by solving for λ and dividing by the volume of the microwells ($V_{PDMS\ array} \approx 0.14\ \mu L$, $V_{PMMA\ array} \approx 1\ \mu L$), we can calculate the concentration of target molecule in our sample:

$$\frac{\ln(P(x = 0))}{-V_{well}} = \frac{\lambda}{V_{well}} = c$$

The theoretical detection range can be calculated by assuming that the lower limit of detection shows 99.9% of all wells to be with no target molecule and for the upper limit having 0.1% of wells with the same condition. By insertion in the above equation, the following limits of detection can be calculated:

$$\frac{\ln(0.999)}{-V_{PDMS\ array}} = c_{PDMS\ array, low} \approx 100\ copies/\mu L$$

$$\frac{\ln(0.001)}{-V_{PDMS\ array}} = c_{PDMS\ array, high} \approx 500,000\ copies/\mu L$$

$$\frac{\ln(0.999)}{-V_{PMMA\ array}} = c_{PMMA\ array, low} \approx 10\ copies/\mu L$$

$$\frac{\ln_{10}(0.001)}{-V_{PMMA\ array}} = c_{PMMA\ array, high} \approx 70,000\ copies/\mu L$$

Table S1 List of parts required for the assembly of the plasmonic POCT dPCR sensor system

Part	Supplier	Amount
Raspberry Pi 4 – 4Gb	Adafruit, USA	1
Differential digital to analog converter ADC 3304	Microchip Technology, USA	1
Luxeon Rebel LEDs LXML-PR02-1000	Lumileds, USA	4
MOSFET, SK C3851 67 Y	SK Hynix, Korea	2
Heatsink BGA35X35X10	Fischer, Germany	1
Resistors 100 / 200 / 750 / 1000 / 10000 Ω	ICBanq, Korea	9
Blue LED	ICBanq, Korea	1
Red LED	ICBanq, Korea	1
Board to Wire connector 5046-02	Molex, USA	2
Stacking Header for Pi - 2x20 Extra Tall Header	Adafruit, USA	1
Header for Raspberry Pi - 2x20 Short Female Header	Adafruit, USA	1
FAN- Pi-FAN LD3007MS	ICBanq, Korea	1
Li-ion Battery PB-PD20	XR-Smart Tech Industry, China	1

Table S2. Sequence of the forward and reverse primers as well as the probe used for dPCR experiments

Name	Sequence 5'-3'
Forward primer	TACAGGGAAGAGGCAATG
Reverse primer	AACATGATGCCCCGAAGCTA
Probe	[FAM]-CCCAGTCAAACCTAAGCAGCGGCTA-[BHQ1]

Table S3. Comparison table of heating systems for PCR.

	Peltier element ^{1, 2}	Resistive heater ³⁻⁵	Plasmonic heating
Heating rate [°C/sec]	2 - 5	5 - 9.6	3.3 - 10.7
Cooling rate [°C/sec]	2.6 - 4	3 - 5	5.5 - 8
Power consumption [W]	48 - 200	2.5 - 20	4.5 - 15
Temperature sensing	Thermocouple	Resistive temperature detector (RTD)	-

References

1. J. Khandurina, T. E. McKnight, S. C. Jacobson, L. C. Waters, R. S. Foote and J. M. Ramsey, *Anal. Chem.*, 2000, **72**, 2995-3000.
2. J. Wang, Z. Chen, P. L. Corstjens, M. G. Mauk and H. H. Bau, *Lab Chip*, 2006, **6**, 46-53.
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5. X. Qiu, S. Ge, P. Gao, K. Li, S. Yang, S. Zhang, X. Ye, N. Xia and S. Qian, *Microsyst. Technol.*, 2017, **23**, 2951-2956.

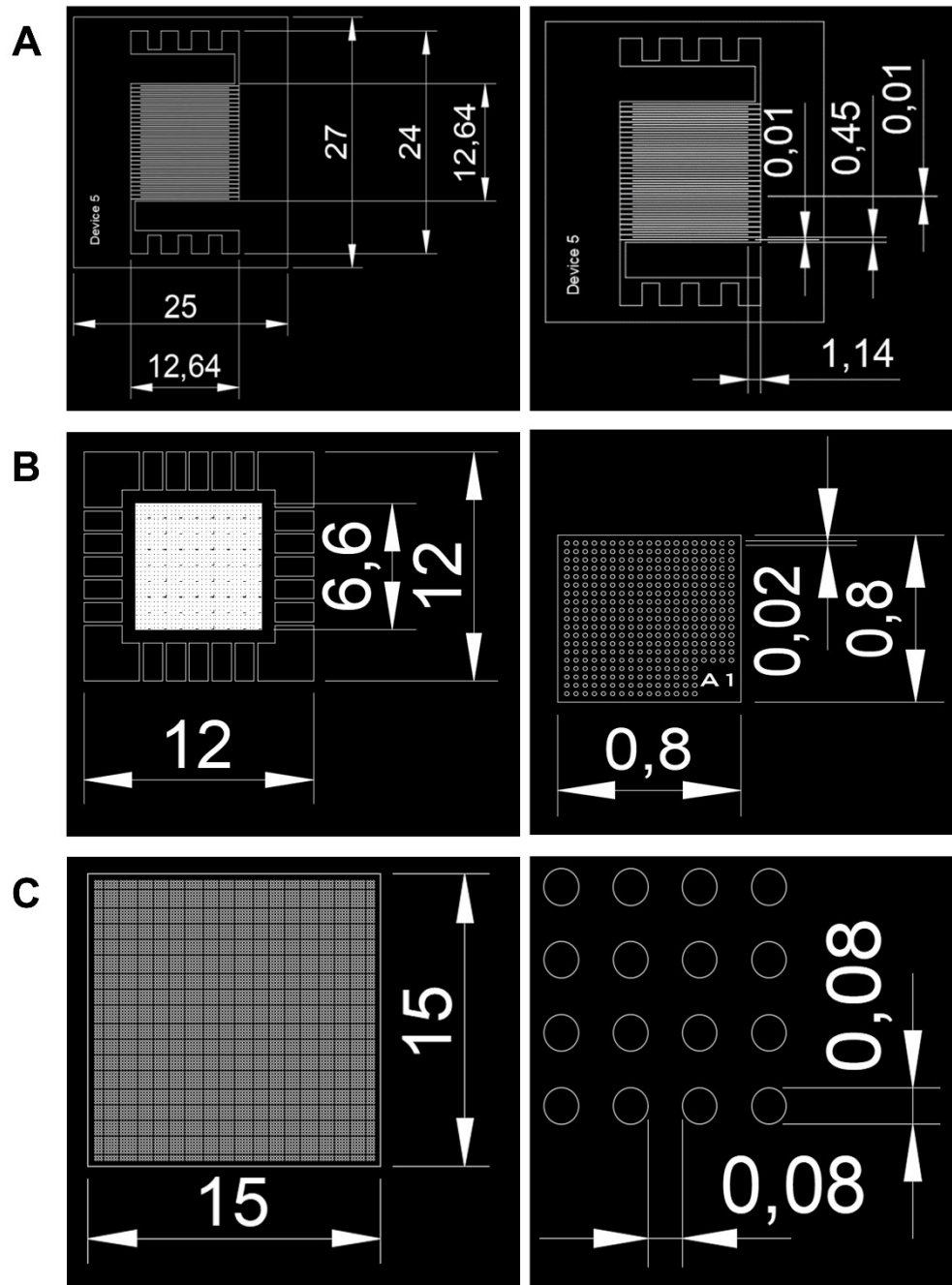


Fig. S1 CAD drawings and dimensions of the gold layer and microwell arrays. CAD drawing of the gold layer with the integrated wire structure and connection pads (A). CAD drawing of the PDMS microwell array consisting of 64 panels with 345, 20 μm diameter microwells each, resulting in a total of 22,080 microwells (B). CAD drawing of the PMMA microwell array consisting of 8,100 microwells with 80 μm diameter (C). All dimensions are mm.

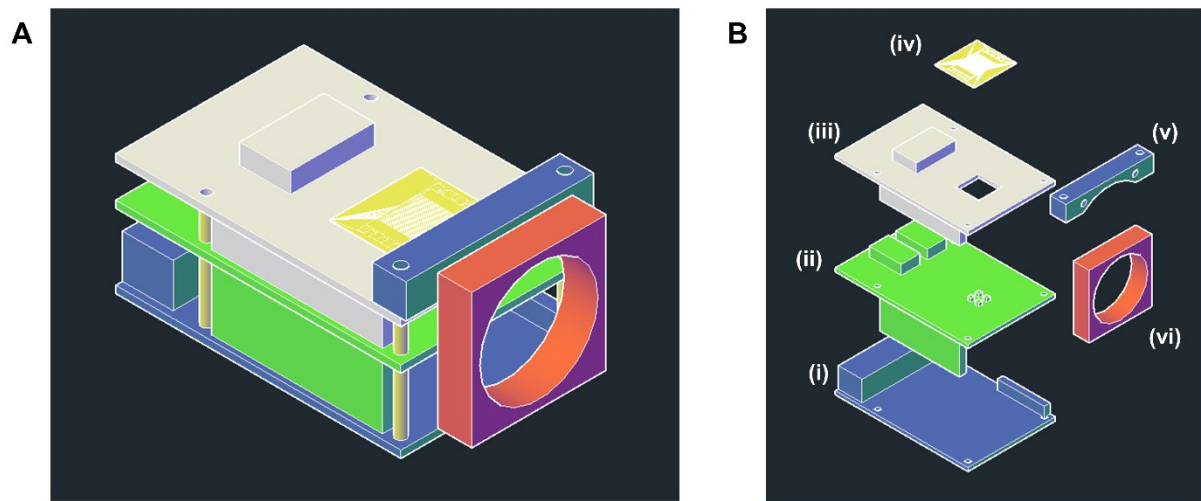


Fig. S2 CAD drawing of the device. CAD drawing of the assembled plasmonic, POCT dPCR sensor system (A). Exploded view of the device showing the Raspberry Pi single board computer (i), the bottom PCB (ii), the top PCB (iii), the gold layer on the glass wafer (iv), the mount for the cooling fan (v), and the cooling fan (vi) (B).

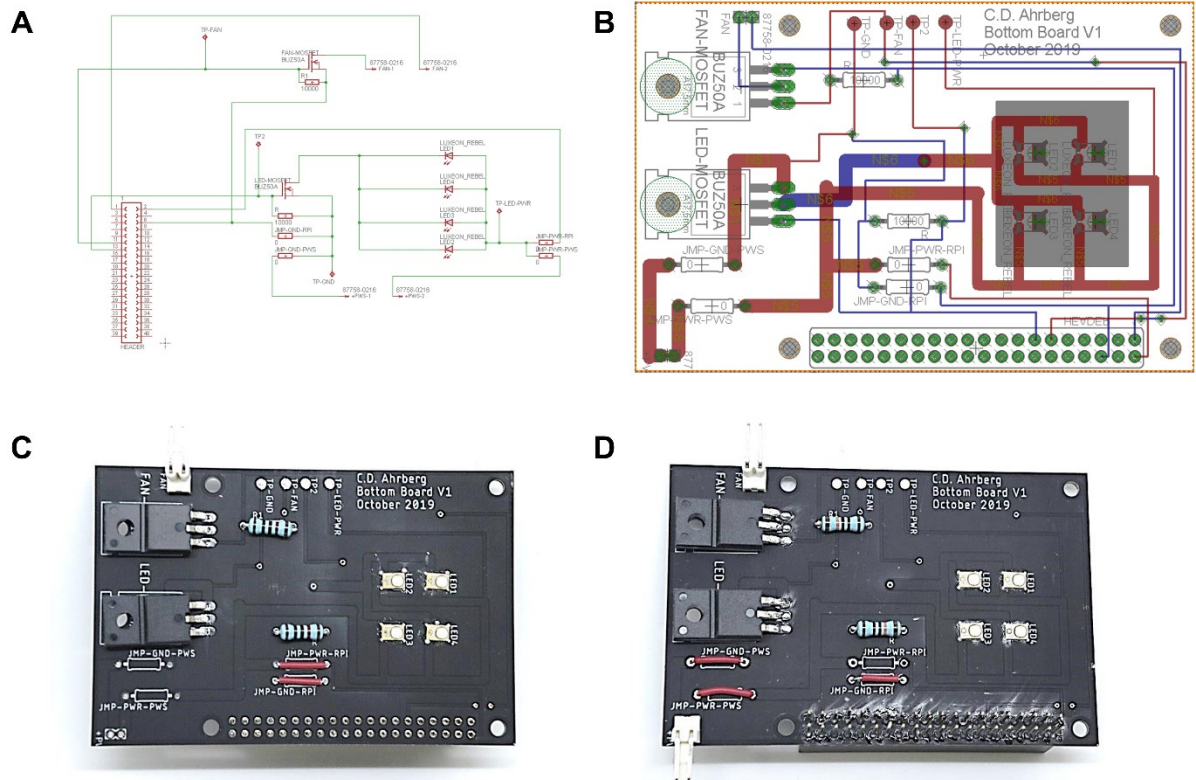


Fig. S3 Bottom PCB assembly. Schematic of the bottom PCB used for mounting the high power LEDs (A) and corresponding board layout (B). Photograph of the assembled PCB with the jumpers in the configuration used for power the LEDs from the Raspberry Pi (C) and the jumper configuration used when using an external power supply for the LEDs (D). For better cooling, a heat sink was mounted on the bottom of the PCB underneath the four high-power LEDs.

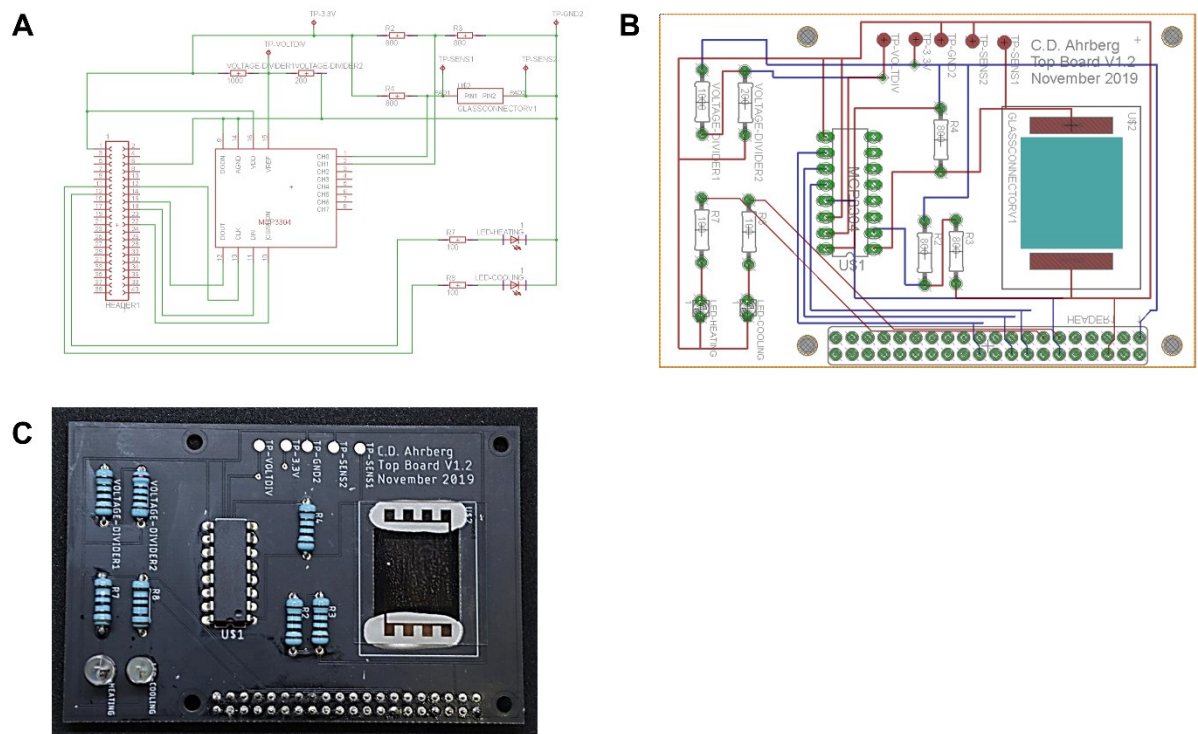


Fig. S4 Top PCB assembly. Schematic of the top PCB used for temperature measurement and mounting of the gold layer (A) and corresponding board layout (B). Photograph of the assembled top PCB (C).

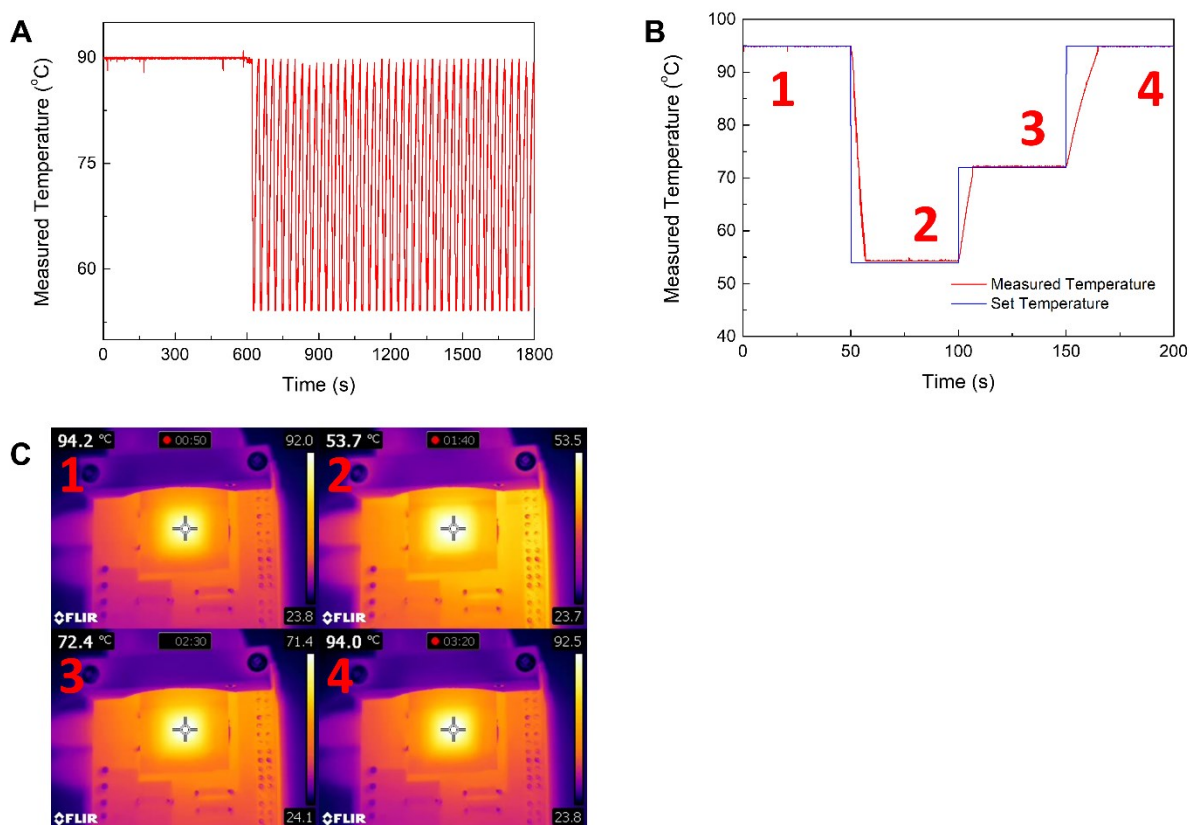


Fig. S5 Thermal cycling results using external power supply for LEDs. Graph showing measured temperature during a PCR run consisting of a 10 minute hot start and 40 denaturation, annealing/extension steps (A). Graph showing set temperature and measured temperature for three different temperatures, the graph shows that the desired temperatures can be stably reached with high accuracy by the external power supply (B). Thermal images taken at different times during the experiment testing the stability (C). Thermal images illustrate that powering the LEDs by an external power supply also results in a homogeneous temperature distribution across the heating area.

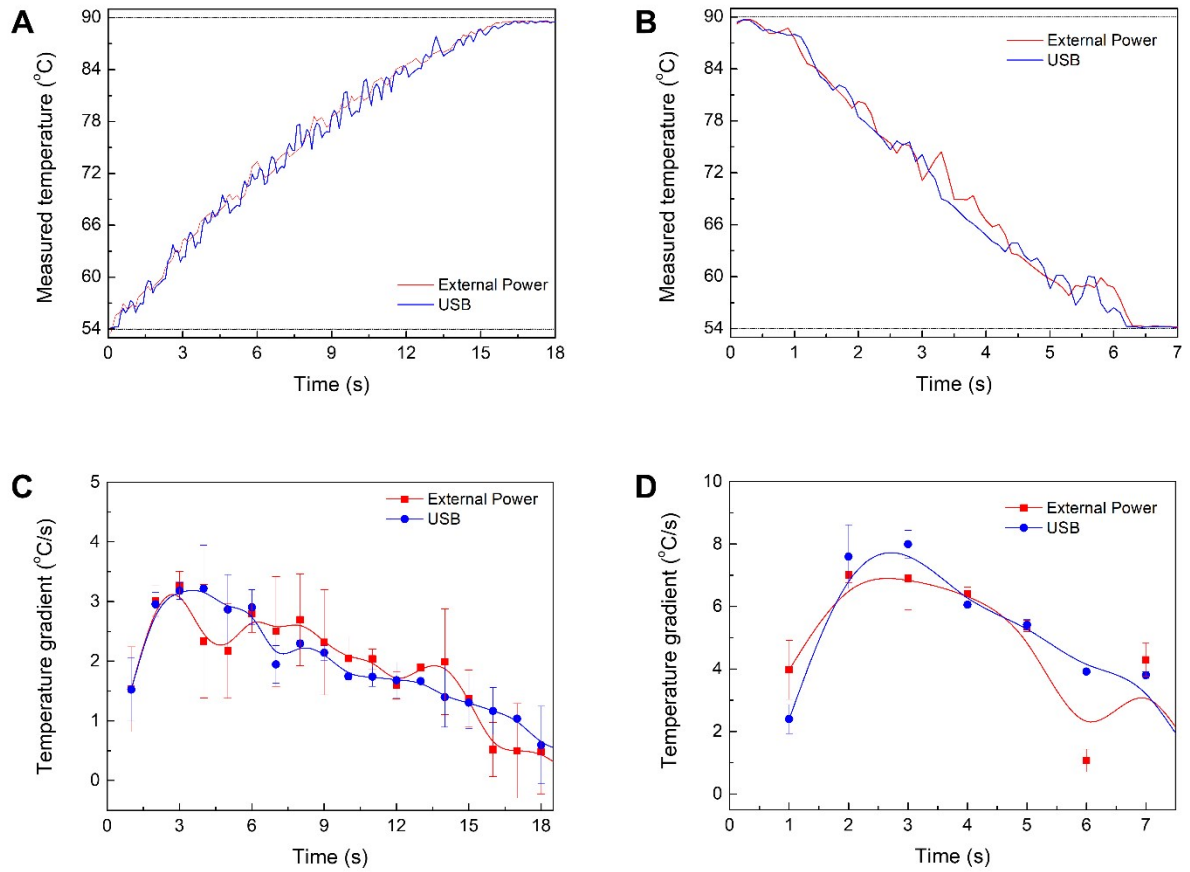


Fig. S6 Comparison of heating and cooling rates between internal and external power supply for powering LEDs. Comparison of temperature development during heating (A) and cooling for LED power supplied by internal power supply and an external power supply (B). Momentary heating (C) and cooling rates for both power options (D). For all values, the average rate over 40 cycles is plotted including 95% confidence interval. Lines indicate the trends in the data.

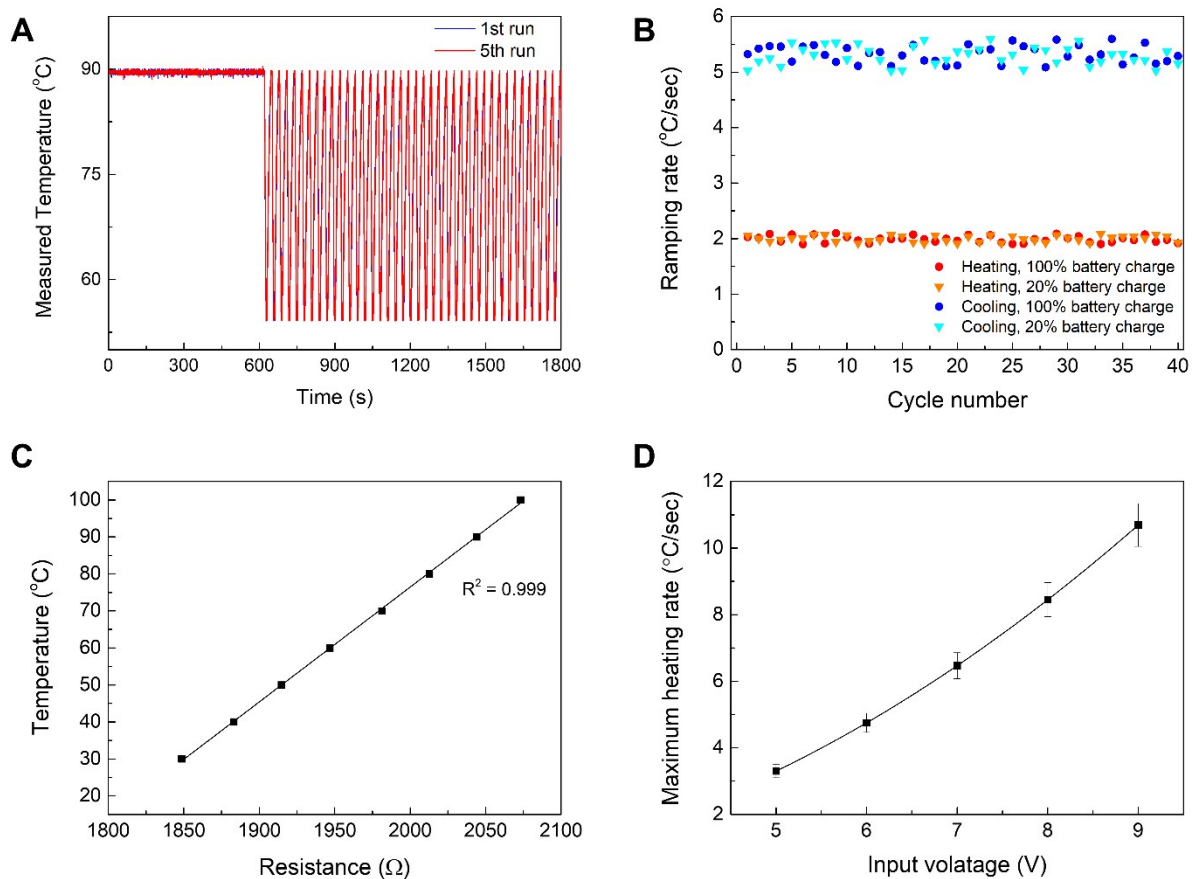


Fig. S7 Heating performance of the plasmonic system. Temperature profile is measured by the device when powered by an external battery and the graph shows the 1st and 5th run on the same battery charge (A). After the fifth PCR experiment, the battery still had 80% charge remaining. Average heating and cooling rates during a PCR run when the device is powered by an external battery (B). The data show that the device can be powered through an external battery. Furthermore, it can be seen that the effects of battery charge on the heating and cooling rate are negligible. Graph of the measured temperature against the resistance of gold layer (C). Graph of maximum heating rate against input voltage (D).

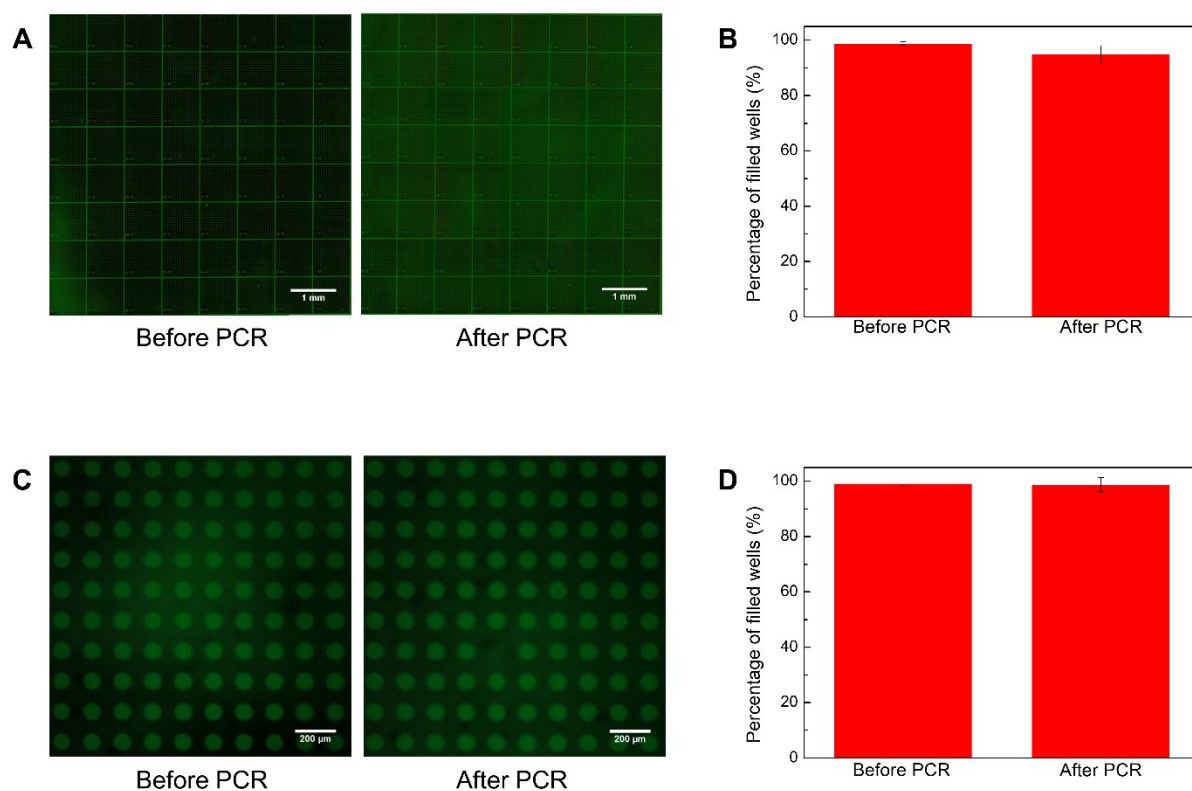


Fig. S8 Filling and evaporation of sample from microwell arrays. Fluorescence images of a PDMS microwell array filled with a fluorescein solution before and after thermocycling (A). Bar diagram showing percentage of filled wells in PDMS microwell array before and after thermocycling, error bars show standard deviation of three separate experiments (B). Equivalent data for PMMA microwell array (C) and bar diagrams of filling and evaporation in PMMA microwell arrays (D). The data shows that the microwells are filled with large efficiency for both materials and that evaporation is low in both cases.

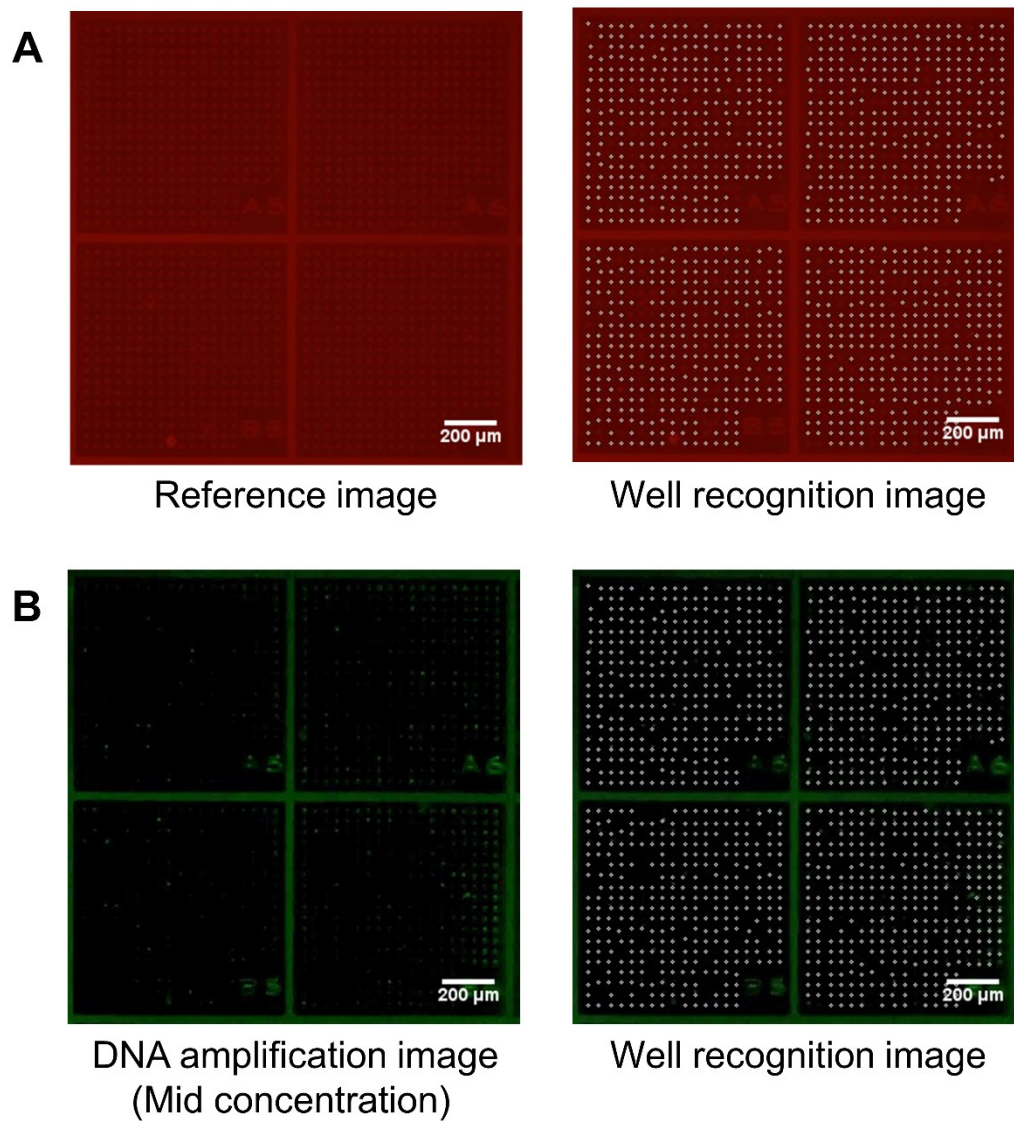


Fig. S9 Illustration of the image analysis algorithm. The image analysis algorithm uses the images recorded of the red ROX reference dye to identify the locations of the filled wells using a Hough algorithm (A). Identified well locations are then transferred to the fluorescence images of the green reporter dye and fluorescence intensity is extracted at the found locations (B).

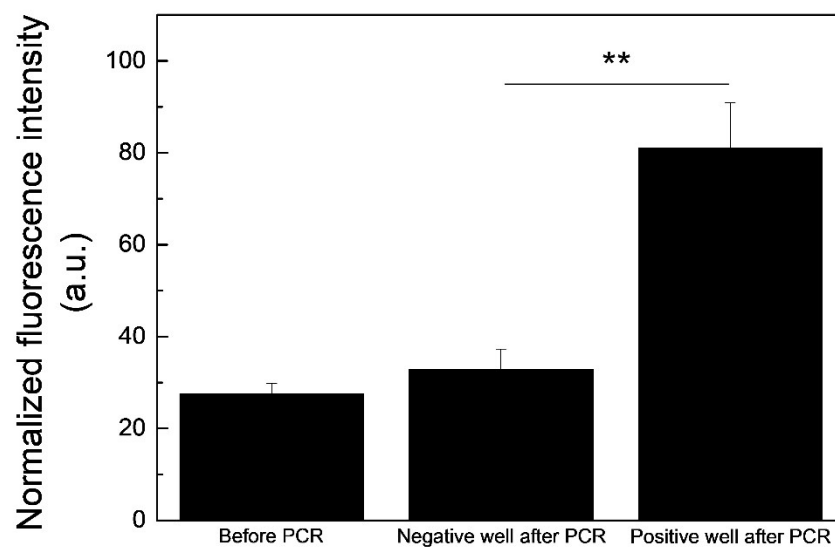


Fig. S10 Graph of the normalized fluorescence intensity of PDMS well arrays (** $p < 0.01$).