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Supporting Information

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Clockwork-PCR Including Sample Preparation

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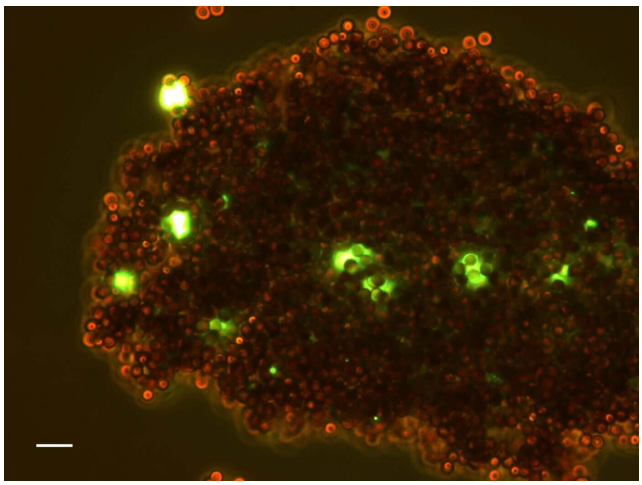
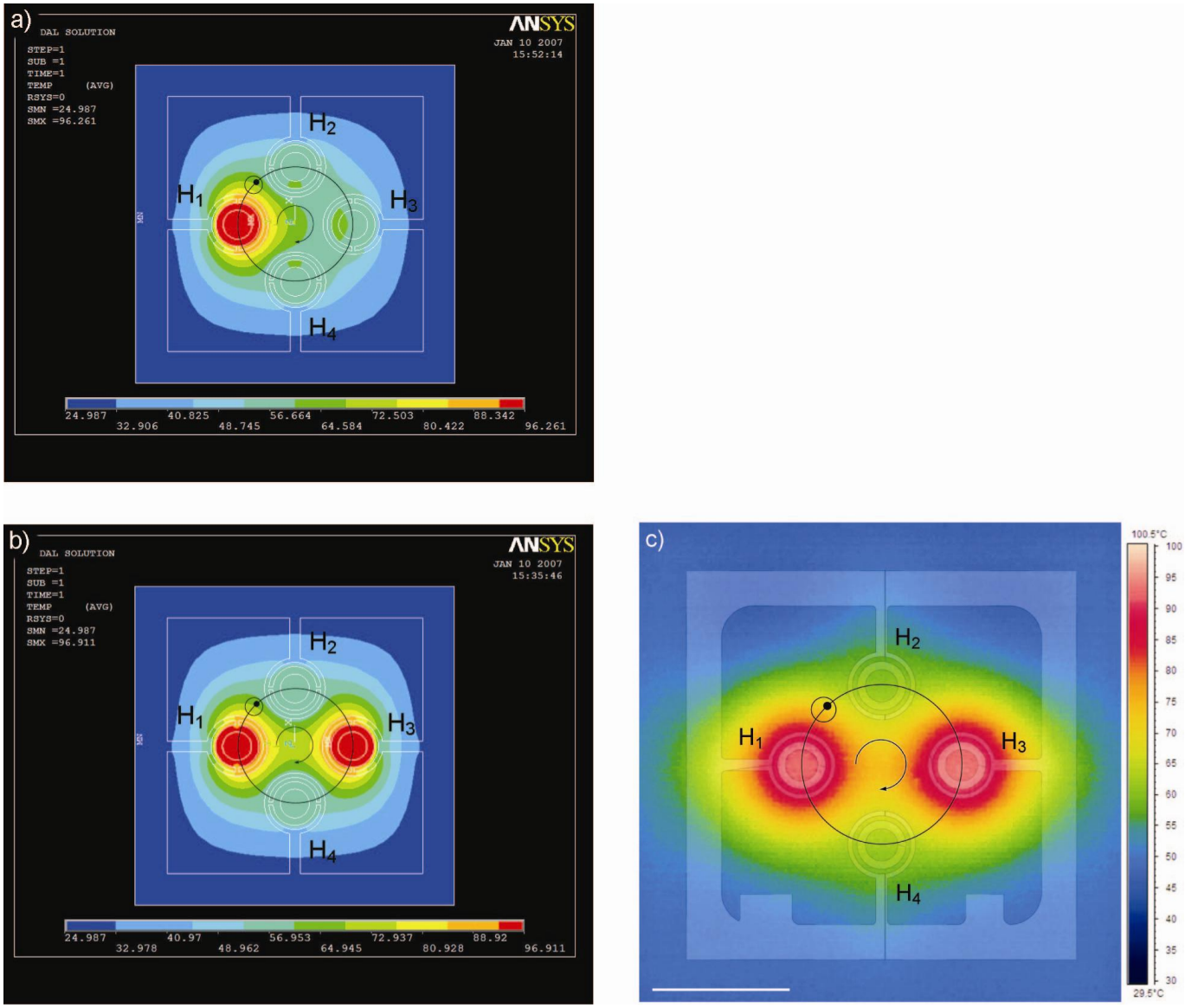
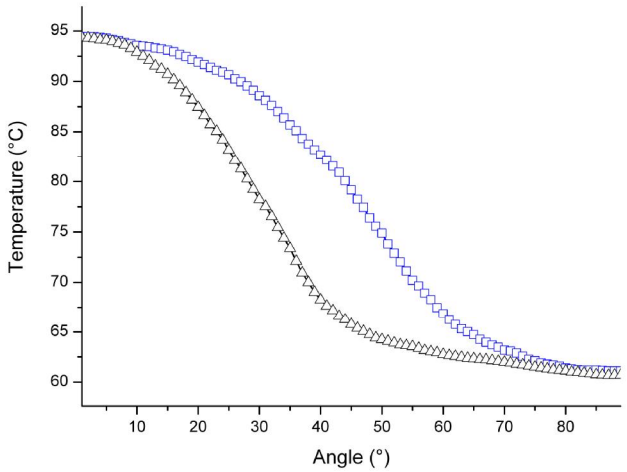


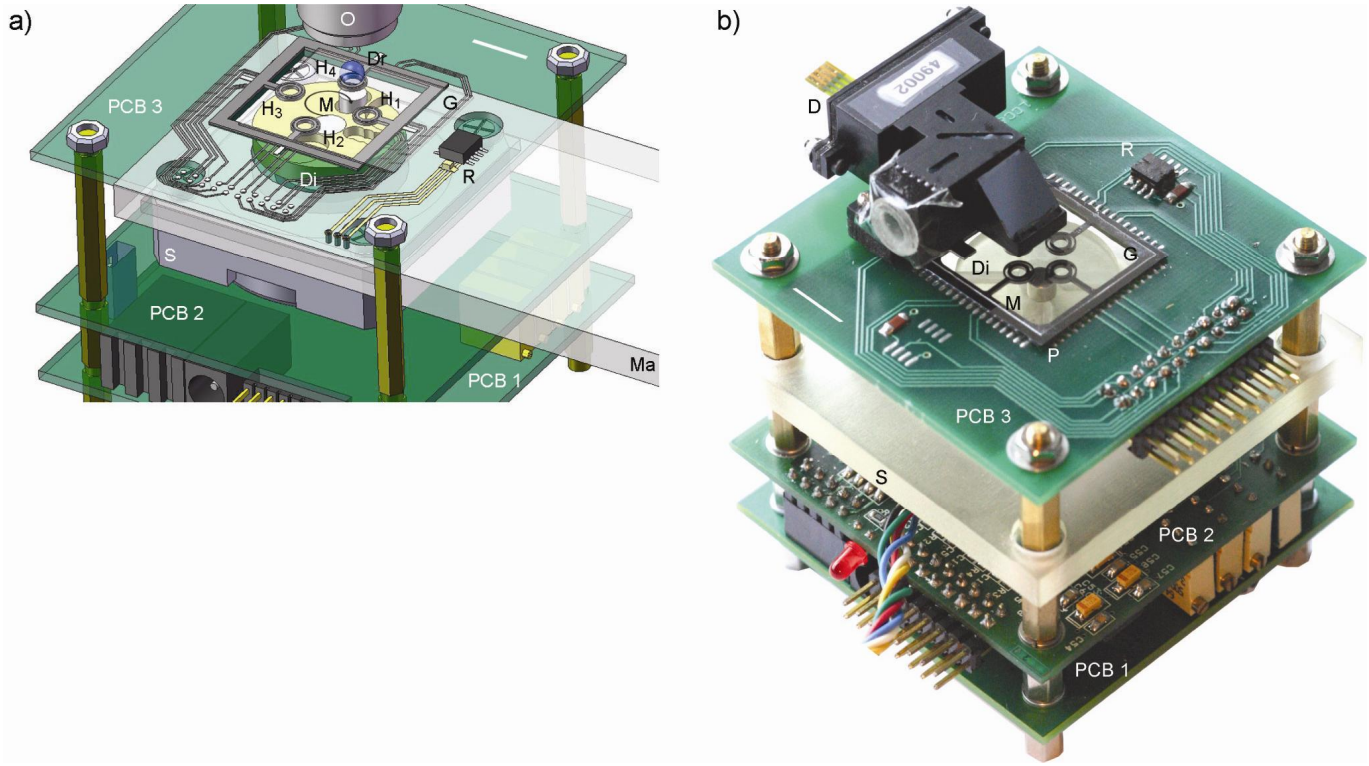
Supplementary Figure 1. GFP-transfected THP-1 cells immunocaptured onto Dynabeads CD15. Most of the cells were buried inside the bulk of the superparamagnetic particles' slurry. Scale bar, 10 μm.



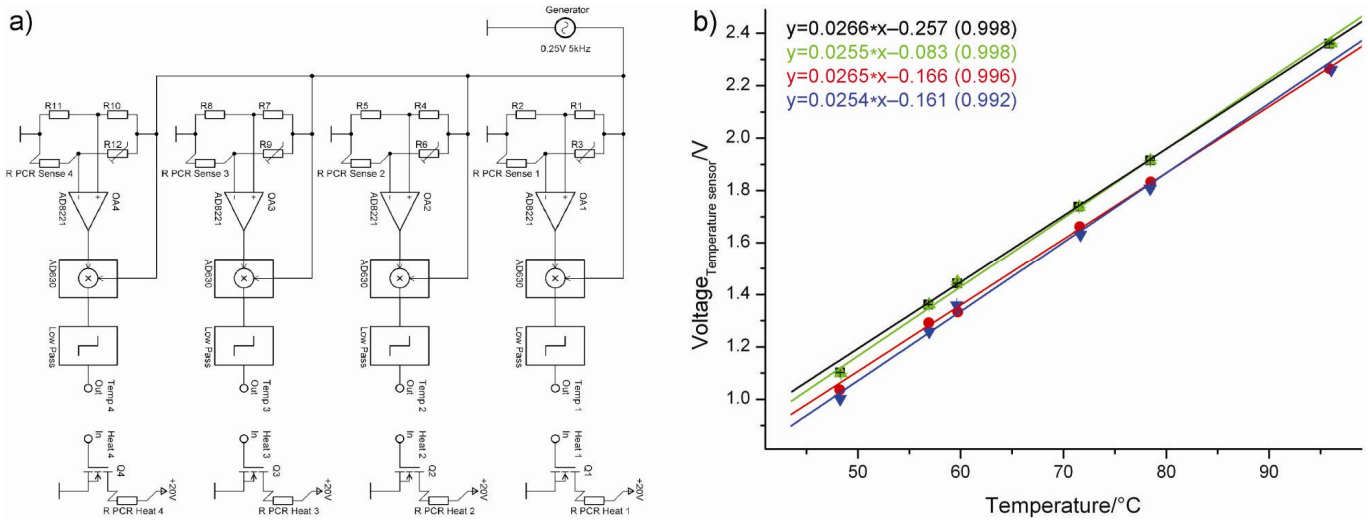
Supplementary Figure 2. Steady-state temperature distribution on the Teflon-coated glass substrate based on the thermocycling protocols 1 and 2 using ANSYS Multiphysics 10 FEA software (ANSYS). The four heaters/temperature sensors, traveling path of the droplet, and droplet containing the Dynabeads CD15/PCR mixture (●) emulsified in mineral oil (○) are superpositioned. a) The mean temperatures within the inner rings are 95.0, 60.0, 60.0, and 60.0 °C for the temperature zones 1–4, respectively. b) The mean temperatures within the inner rings are 95.0, 60.0, 95.0, and 60.0 °C for the temperature zones 1–4, respectively. c) IR-image of the temperature distribution on the Teflon-coated glass substrate based on the thermocycling protocol 2. The mean temperatures within the inner rings are 94.4, 60.5, 95.1, and 60.8 °C for the temperature zones 1–4, respectively. Scale bar, 10 mm.



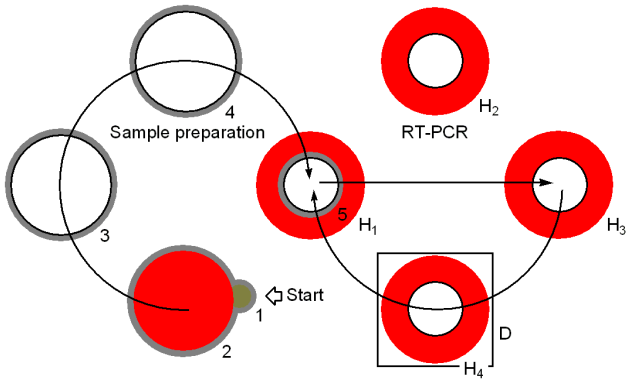
Supplementary Figure 3. Temperature transition between the temperature zones 1 and 2 along the traveling path of the droplet extracted from figure 2 and supplementary figure 2c. a) The open triangles show the thermocycling protocol 1. b) The open squares represent the thermocycling protocol 2. A rotation of 90 ° translated into a traveling distance of 5.8 mm.



Supplementary Figure 4. a) Schematic of the set-up and b) photograph of the prototype hand-held unit. PCB 3 printed circuit board 3; R TSic-706-reference temperature sensor (Innovative Sensor Technologies) for calibrating the PCR chip; H_1–H_4 heaters/temperature sensors 1–4; G Teflon-coated glass substrate; P PCR chip; Dr rotating droplet containing the template DNA and RT-PCR mixture; M permanent magnet glued to a Di rotating disc; S stepper motor fixed to a Ma macroscopic x, y-stage for coupling a circular and linear movement; O long distance objective of a fluorescence microscope coupled with a photomultiplier tube. D miniaturized fluorescence detector.[26] Real-time monitoring was accomplished while the droplet paused for 1–4 s in the focus of the objective in the extension zone 4. Other PCBs contain the microelectronics for the thermal management, optics, and power supply. Scale bar, 10 mm.



Supplementary Figure 5. a) Microelectronics schematic of the PCR chip. Up to four heaters were independently controlled. b) Calibration of the PCR chip. The voltage outputs of every temperature sensor for three separate measurements were plotted versus the temperature. The solid lines are linear regression fits to the voltage outputs of the four temperature sensors with y=B∗x+A (correlation coefficient). Error bars correspond to the standard deviation. During the calibration, the top PCB accommodating the PCR chip and TSic-706-reference temperature sensor was immersed into FC-70 Fluorinert (3M) at different temperatures between 40–100 °C.



Supplementary Figure 6. Trajectory for the first thermocycle. After completing the cell lysis in the temperature zone 1, the droplet was first linearly moved to the temperature zone 3, and then rotated clockwise back to 1 via 4. This approach enables a simple microfluidic actuation by coupling a circular and linear movement. Otherwise, a rotation has to be superpositioned with a two-dimensional movement to complete of a full rotation for the very first thermocycle. a) For thermocycling protocol 1, the heater(s) 1 and 2–4 were at 95 and 60 °C, respectively. Since the linear movement takes 3 s, the thermocycling profile is barely distorted. b) For thermocycling protocol 2, the heaters 1 and 3 as well as 2 and 4 were at 95 and 60 °C, respectively. 1 Dynabeads CD15; 2 blood droplet spiked with GFP-transfected THP-1 cells; 3 and 4 washing solution droplets; H_1-H_4 heaters/temperature sensors; D fluorescence detector.

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Supplementary Movie 1

Supplementary Movie 1. Sample preparation. Isolation of THP-1 cells from 100 nL blood using 100 nL Dynabeads CD15 in real-time. In this experiment, the concentration ratio of the Dynabeads CD15 and CD15-expressing cells is 7500:1, which allows for an extremely fast isolation. After extraction, the surface-bound THP-1 cells were washed two times in 25 μL washing solution to remove residual contaminants, which interfere with the RT-PCR.

Supplementary Movie 2

Supplementary Movie 2. Thermocycling protocol 1. The residence times of the droplet in the temperature zones 1–4 were 1 s each. With transition times between adjacent temperature zones of 1 s each, the droplet completed one thermocycle in 8 s. One full rotation translated into a travelling distance of 23.2 mm.

Supplementary Movie 3

Supplementary Movie 3. Thermocycling protocol 2. The residence times of the droplet in the temperature zones 1and 3 as well as 2 and 4 were 1 and 4 s, respectively. With transition times between adjacent temperature zones of 1 s each, the droplet completed one thermocycle in 7 s. One full rotation translated into a travelling distance of 23.2 mm.