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

Malaria detection using inertial microfluidics

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Supplementary information

RT-PCR and gel analysis

Cells were concentrated into 200 μL of PBS and processed with the Malaria Kit (Norgen, # 34800, Canada) according to the manufacturer's instructions for DNA extraction and purification. Real-time PCR was performed using a Step One Plus^{TM} Detection System (Applied Biosystems, Foster City, CA) using SYBR Green (Invitrogen). PCR reactions were performed in a 96-well optical plate, with cycle conditions as follows: 95°C for 3 min, followed by 40 cycles of 94°C for 15 s and 60°C for 30 s. The threshold cycle (CT) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. CT values data were obtained using default threshold settings. The melt curve analysis was obtained between 65° to 95°C with a reading taken every 0.1°C after holding the temperature for 5 s. For demonstration of primer specificity, purified DNA is subjected to PCR using thermal cycler (Bio-Rad, USA) according to manufacturer's instructions. Amplified PCR reactions are loaded on a 1X TAE 2% Agarose DNA gel (stained with 1X GelRed) (all Sigma) along with 10 μL ladder supplied by Norgen kit, and resolved at 100V for 45 min. Gel is imaged with the Bio-Rad machine under exposure times optimized for intense bands.

Table S1. The range of flow rates at which WBCs are focused for different device designs.

Designs of microfluidic devices		Flow rate (µL/min)
30 µm / 90 µm	1:1:1	100 - 400
	1:2:1	100 - 300
40 µm /120 µm	1:1:1	100 - 200
	1:2:1	50 - 100

Table S2. Tabulation of optimal *Re* value.

Speed (µL/min)	*Re* (based on 30 µm width in contraction array)
100	25.64
200	51.28
400	102.56
500	128.20

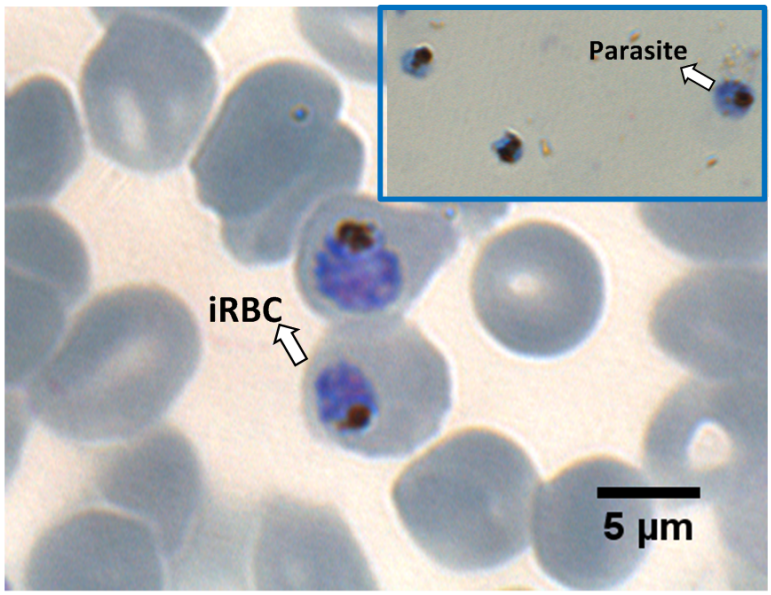

Figure S1: Optical microscopic images showing infected RBCs (iRBCs) before lysis and malaria parasites (the inset picture) after lysis using low concentration of saponin. Thin Giemsa smear was performed to check that the iRBCs used contained single parasite. This was to ensure that the spiking dosages were accurate and clinically relevant (i.e., low level parasitemia) where 2k-10k of malaria parasites are expected to exist in 1 mL of blood.

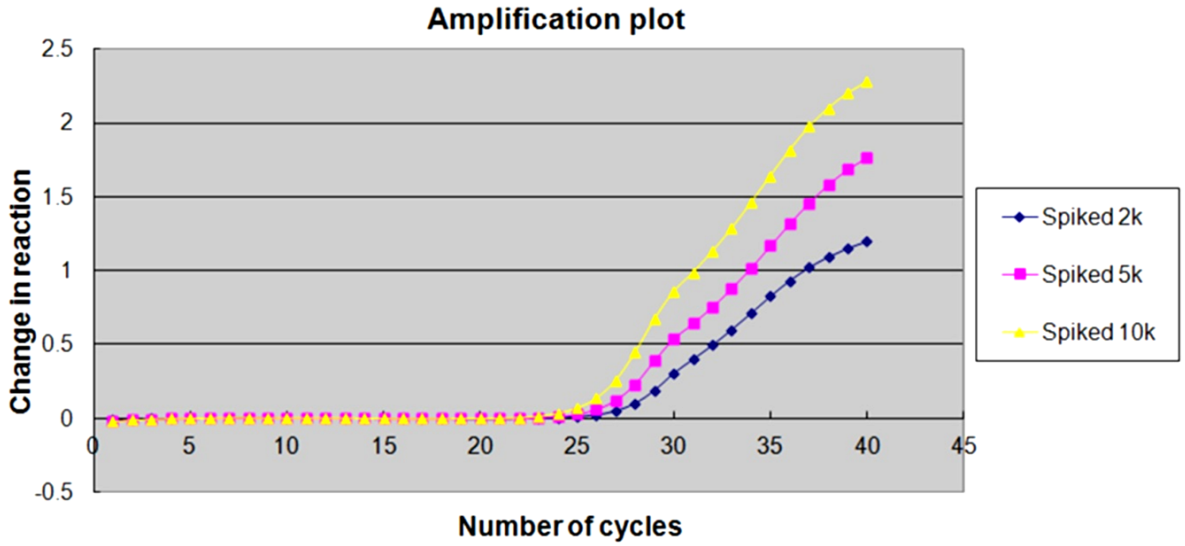

Figure S2: Calibration curve generated with qPCR data for estimation of parasite recovery.

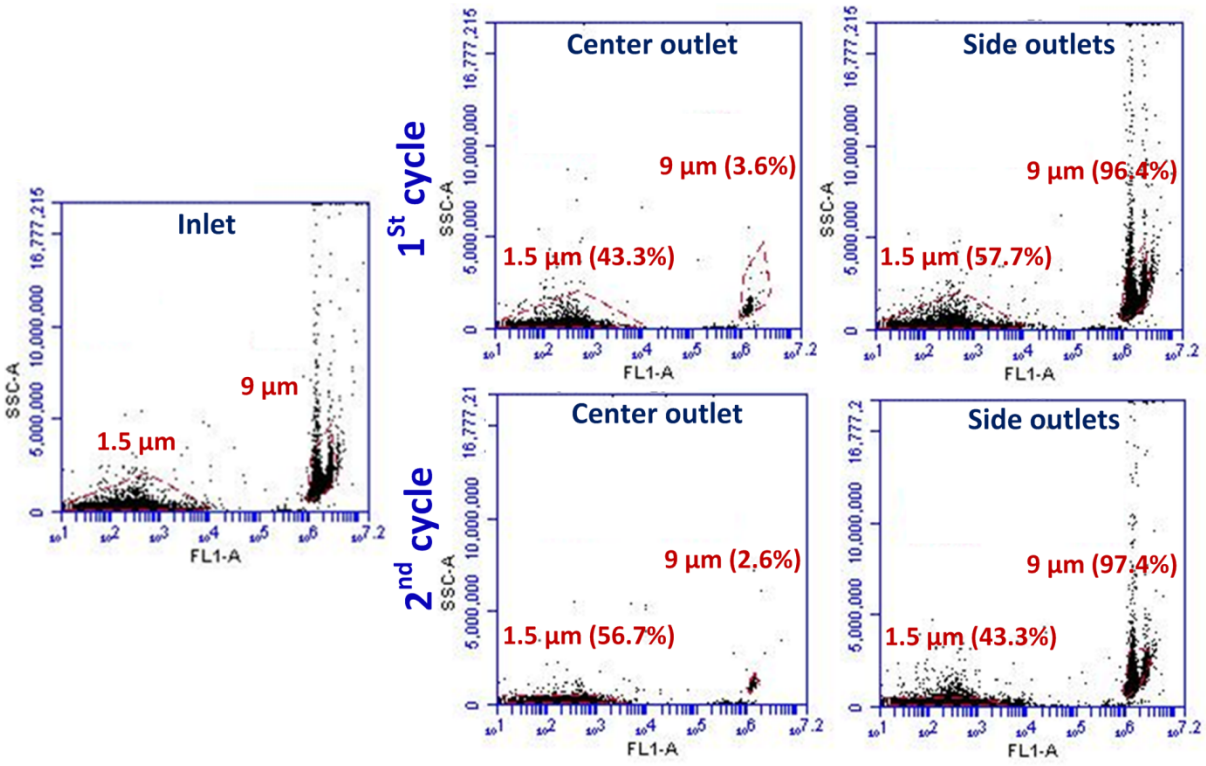

Figure S3: Scatter plots captured using flow cytometer (Accuri C6, BD Biosciences, USA) showing the results of separations of particle mixtures in a microfluidic device (30/90 design, 1:1:1).

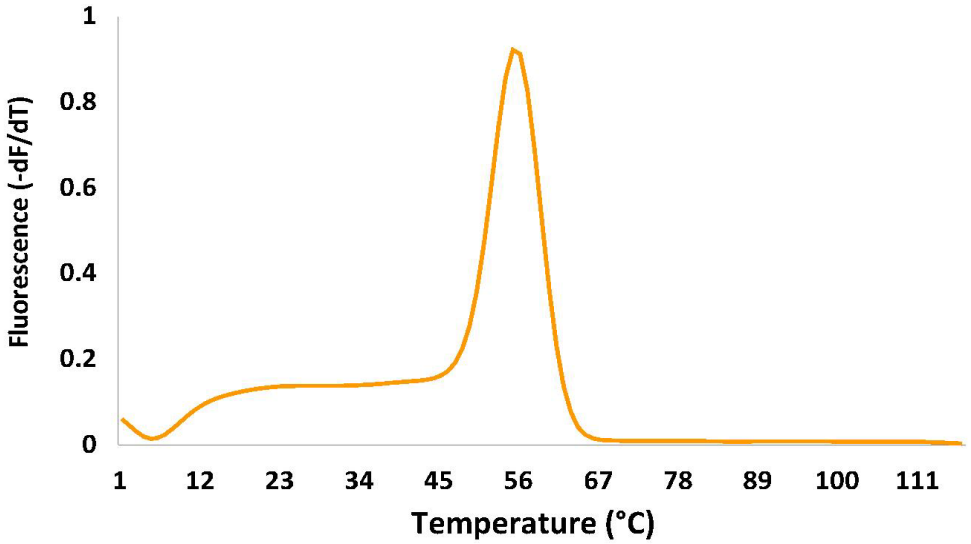

Figure S4: Melt curve profile to demonstrate specificity of primers used in detection of malaria parasites.

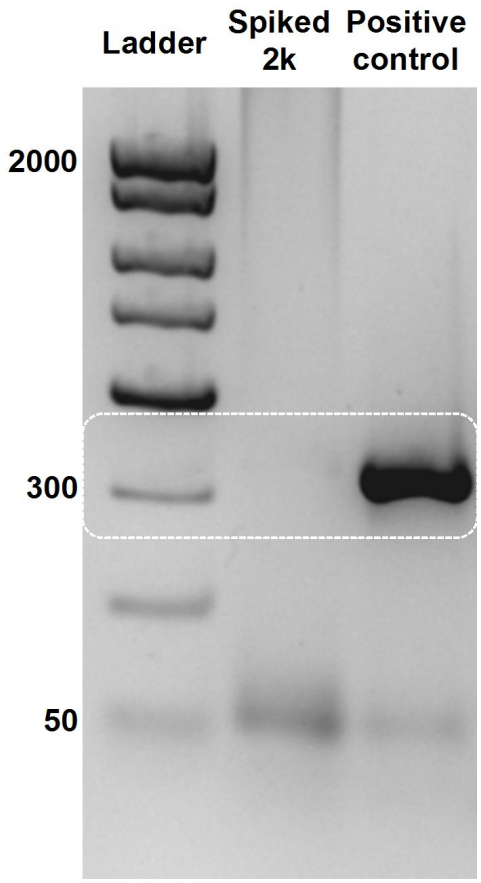

Figure S5: A GelRed-stained 1X TAE 2% agarose gel showing the DNA bands after PCR amplification. A single band at 350bp for the positive control was observed, which corresponds to the malaria target amplicon. No amplification of the target is observed in the

spiked 2k sample, demonstrating the difficulty in detecting low quantity of malaria parasites in whole blood samples.

SI Movie Legends

Movie S1

High speed video (6400 fps) illustrating the complete (near 100%) removal of 9 μm beads (10^4/mL) from side outlets using 30/90 μm device at flow rate of 400 μl/min (*Re* ~ 102). Unfocused 1.5 μm beads (10^5/mL) are dispersed in the entire channels exiting from all outlets.

Movie S2

High speed video (6400 fps) illustrating the complete isolation of white blood cells (at 0.25x concentration (~ 25×10^3 cells/mL)) at the device outlet *(Re* ~ 102). Malaria parasites are too small to be visualized in the video using bright-filed microscopy.