Supplementary Information

High-throughput and label-free parasitemia quantification and stage differentiation for malaria-infected red blood cells

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Supplementary Text

The equivalent circuit model for the sensor.

The corresponding equivalent circuit model is presented in Supplementary Information (Figure S1). The loading channel is equivalent to a resistor R_{Salt} and a capacitor C_{PDMS} connected in parallel[1]. The electrical resistance of the microfluidic sensor is mainly dominated by the micropore constriction (i.e., R_{gap} is much larger than other resistances in Figure S1). When an RBC (including membrane capacitance C_{cell} and cytoplasm resistance R_{cell})[2,3] squeezes through the micropore, an ionic current dip occurs due to the reduced conduction cross-section.

Validation of the device and testing apparatus using microbeads

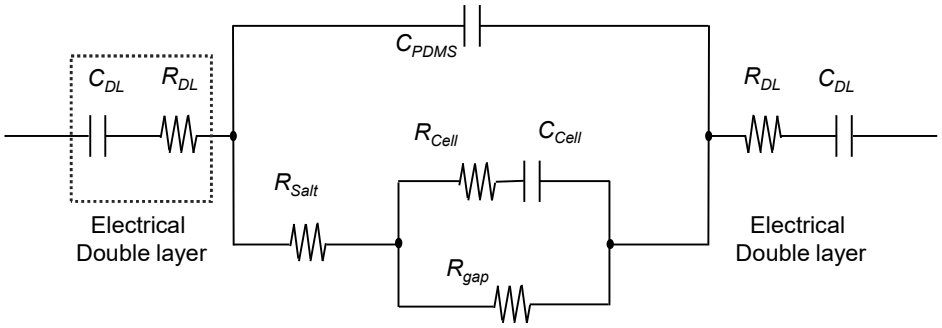
A mixture of polystyrene microbeads with diameters of 5 μm and 10 μm (with a concentration of 1×10^5/ml and 3×10^4/ml, respectively) were used to validate the microfluidic device and the testing apparatus (Supplementary Information Figure S2). Figure S2a shows the current trace for the mixed microbeads. Figure S2b shows the scatter plot and the histogram of the transit time versus current dip amplitude (ΔI). Two separate populations with mean ΔI values of 0.09 nA and 0.7 nA can be clearly observed, representing the two different sized microbeads. The events accounts of the two groups are 1681 and 4853, respectively. The percentage of each polystyrene beads measured from the device was consistent with the pre-mixed ratio. This confirmed the functionality of the microfluidic device and the testing apparatus.

Blind test: 0.1% Pf-iRBCs

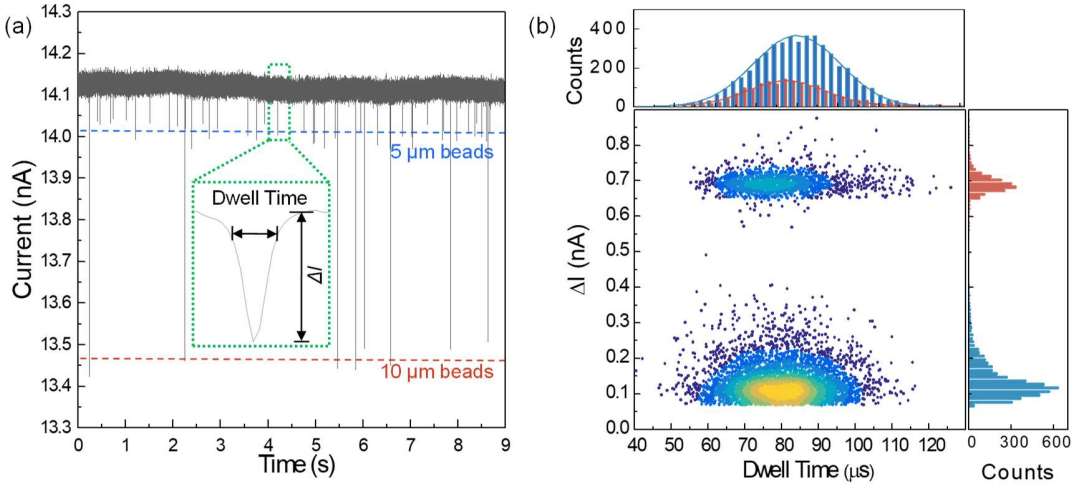
Supplementary Table 1 summary of the overall performance microfluidic sensor as compared to blood-smear microscopy, conducted blindly for 50 samples. As many as 50 fields (each >100,000 RBCs) were checked for blood smear. The scattering plot of the transit time and current dip for these 50 samples were shown in Figure S3. For each sample, it was deemed as positive in the microfluidic deformability sensor when the amount of the points larger than the threshold transit time was bigger than 100. Otherwise, it was regarded as negative. The variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN). If the RBCs were infected, and the transit time had a relative large population above threshold, it was considered as true positive. Otherwise, if the

measured transit time was below the threshold, as compared to the hRBCs, it was considered as true negative. False positive occurred if the RBCs was detected with infection but had not been infected by us. False negative occurred if the RBCs was detected as no infection but was actually infected.

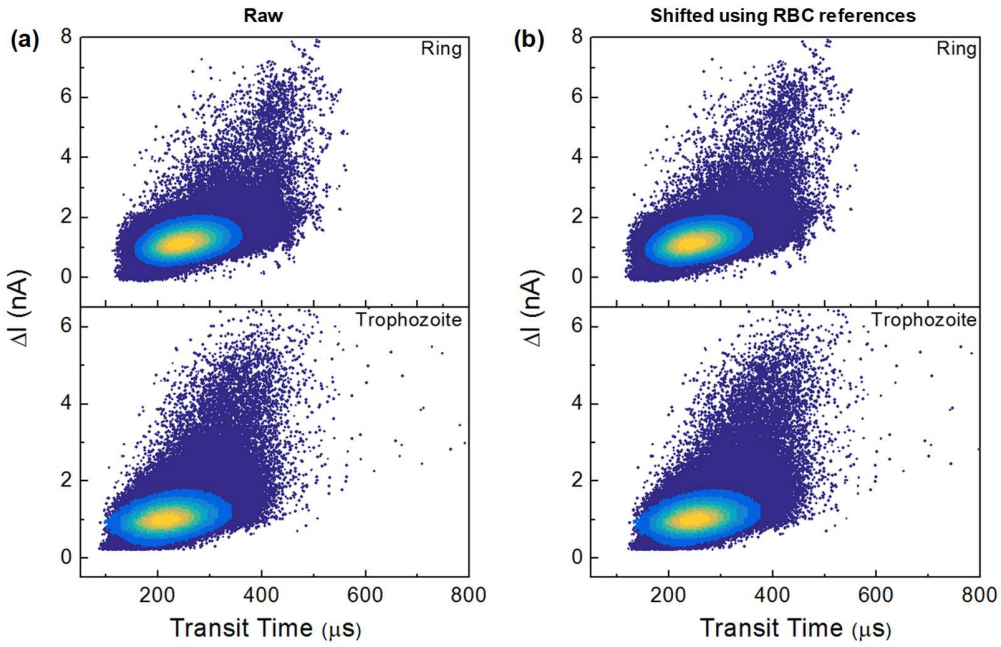
Supplementary Figure



Supplementary Figure 1. The equivalent circuit model for the sensor.



Supplementary Figure 2. Validation of the device and testing apparatus using microbeads. (a) Current traces measured with the mixed microbeads of 5 μm and 10 μm in diameters. (b) The scattering plot and the corresponding histogram for the measurement.



Supplementary Figure 3. Transit time distribution for ring and trophozoite stages at 3% parasitemia. (a) raw data, (b) shifted data using healthy population as reference.

Supplementary Table

Supplementary Table 1. The archived results for the 50 samples using microfluidic sensor as compared to the blood-smear microscopy.

			Microscopy						Microscopy		
Sample	Record	Sensor	T1	T2	T3	Sample	Record	Sensor	T1	T2	T3
1	N	N	N	N	N	26	P	P	P	P	P
2	N	N	N	N	N	27	P	P	P	P	P
3	N	N	N	P	P	28	P	P	P	P	P
4	P	P	P	P	P	29	P	P	P	P	P
5	P	P	P	P	P	30	P	P	P	N	N
6	P	P	P	P	P	31	P	P	P	P	P
7	P	P	P	P	P	32	P	P	P	P	P
8	P	P	P	P	P	33	P	P	P	P	P
9	P	P	N	P	P	34	P	P	P	P	P
10	P	P	P	P	P	35	P	P	P	P	P
11	P	N	P	P	P	36	P	P	P	P	P
12	P	P	P	P	P	37	P	P	P	P	P
13	P	P	P	P	P	38	N	N	N	N	N
14	N	N	N	N	N	39	N	N	N	N	N
15	N	N	N	P	P	40	N	P	N	N	P
16	P	P	P	P	P	41	N	N	N	N	N
17	P	P	P	P	P	42	N	N	N	N	N
18	P	P	P	P	P	43	N	N	N	N	N
19	P	P	P	P	N	44	P	N	P	P	P
20	N	P	N	N	N	45	P	P	P	P	P
21	N	N	N	N	P	46	P	P	P	P	P
22	N	N	P	N	N	47	P	P	P	P	P
23	N	N	N	N	N	48	P	P	P	P	P
24	P	P	P	P	P	49	P	P	P	P	N
25	P	P	P	P	P	50	P	P	P	P	P

Supplementary Table 2. Comparison for various methods.

	Microscopy	**Flow cytometry**	**Real-time deformability cytometry ***	**Micromagnetic resonance relaxometry ****	**This work**
Throughput	>5 mins/slide	High	~100 cells/s	Bulk measurement	500 cells/s
Cost	Low	High	Low	Low	Low
Parasitemia (quantitative)	Yes	Yes	NA	Yes	Yes
Stage determination	Yes	NA	NA	Yes	Yes
Label-free	No	No	Yes	Yes	Yes
Single cell	Yes	Yes	Yes	No	Yes

* Nature Methods, 2015, 12, 199-202
** Nature Medicine, 2014, 20, 1069-1073

Reference

1. J. A. Chen, Y. Zheng, Q. Y. Tan, Y. L. Zhang, J. Li, W. R. Geddie, M. A. S. Jewett and Y. Sun, *Biomicrofluidics*, 2011, **5**.
2. T. Sun and H. Morgan, *Microfluid Nanofluid*, 2010, **8**, 423-443.
3. K. C. Cheung, M. Di Berardino, G. Schade-Kampmann, M. Hebeisen, A. Pierzchalski, J. Bocsi, A. Mittag and A. Tarnok, *Cytom Part A*, 2010, **77a**, 648-666.