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

Supplementary information

A Star Shaped Acoustofluidic Mixer Enhances Rapid Malaria Diagnostics via Cell Lysis and Whole Blood Homogenisation in 2 Seconds.

Section S1. Exposure time measurement

The exposure time was calculated considering the time that fluid travels through the thickness of the entire silicon structure (Fig. S1) at a given flow rate:

$$Exposure\ time = t/U$$

where t and U are thickness of the silicon wafer and fluid velocity before disk shape profile, respectively.

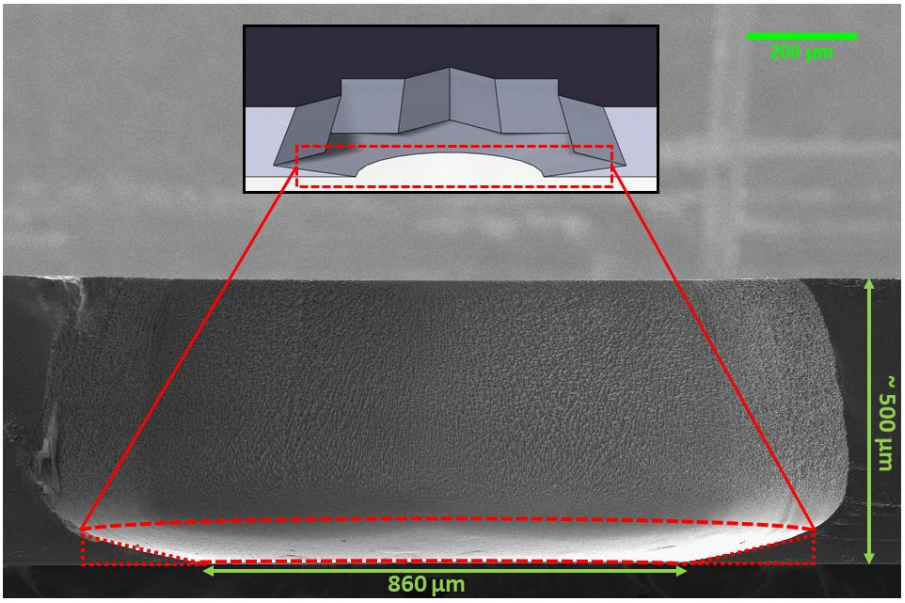

Figure S1. Cross-section SEM of the star shaped profile. Inset: Schematic of cross-section view of the star shaped device.

The fluid velocity was calculated by dividing the flow rate by the surface area that fluid travels from the bottom e.g. inner diameter of the disk shape profile (Figure S1). The thickness and inner diameter of the disk shaped profile were measured by SEM imaging using FEI Nova NanoSEM 430 Instrument at current of 56 pA and voltage of 5 kV.

Section S2. Simulation results in presence of flow rate

Figure S2 presents the streaming and shear rate distribution at 50 µL.min^{-1}. Compared to Figure 3, the streaming and shear rate distribution do not change dramatically when the flow rates are increased to 50 µL.min^{-1}. The highest velocities and shear rates are focussed near the sharp edge of the oscillating disk as well as corners of the star shaped pattern. However, increasing the flow rate reduces the cells' exposure time to the streaming fields, which will reduce the lysing efficiency. At substantially

high flow rates, the back flow pressure exerted on the oscillating structure is also expected to increase the damping and reduce the device performance.

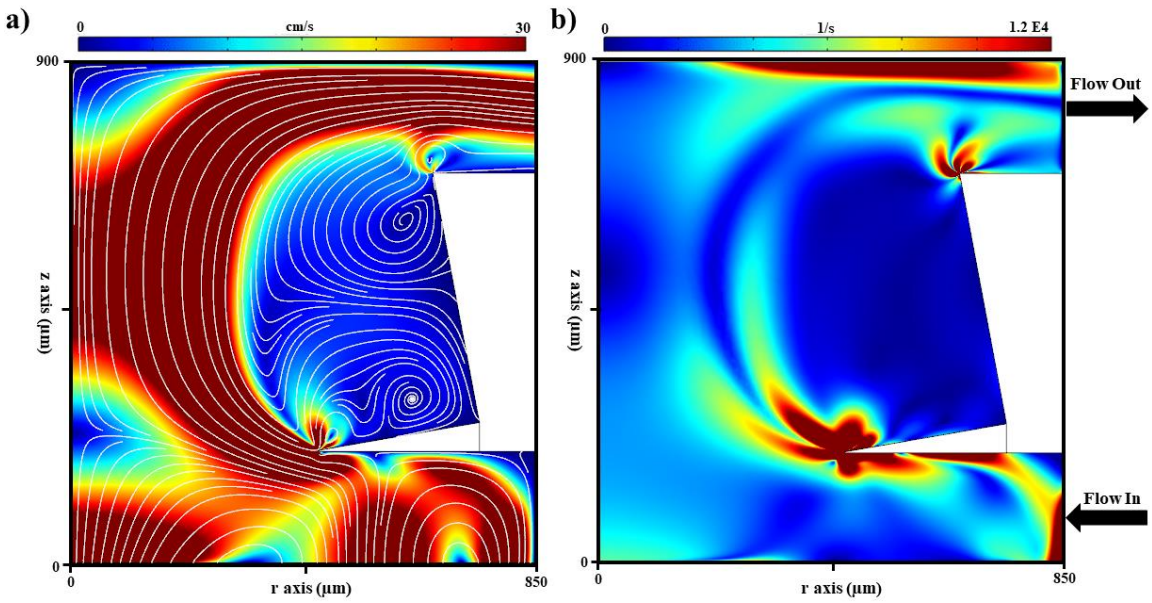

Figure S2. a) Streaming velocity and b) Shear rate distribution in the presence of flow rate (50 µL.min^{-1}).

Section S3. White blood cells (WBCs) lysis

Whole blood (procured from Australian Red Cross) was fractionated by centrifuging at 1500-2000 X g for 10-15 min at room temperature. The centrifugation separates the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs. The upper layer was aspirated using a serological pipette without disturbing the WBC layer. After the complete removal of the plasma layer, the exposed WBCs were carefully aspirated using a plastic transfer pipet in a 2 ml Eppendorf tube. The collected WBCs (approximately 0.5 mL) were then washed thrice in saline (0.9% NaCl solution, Baxter, U.S.A) solution to remove any contamination from the RBCs. 100 µL of cells were dispensed into a new Eppendorf tube and 400 µL 0.4% Trypan Blue (Catalogue no: 15250061, Gibco™) added to make a final concentration of 0.32% and gently mixed. 100 µL of the Trypan Blue-treated cell suspension was transferred to the haemocytometer by allowing the cell suspension to be drawn out by capillary action. Prior to use, the haemocytometer and coverslip were thoroughly cleaned with 80% ethanol to remove contamination. The trypan blue was used to distinguish the live cells from the dead cells by using a microscope, focused on the grid lines of the haemocytometer with a 10X objective and counting the live, unstained cells. The cells/µL were calculated by the following formula.

$$\text{No. of cells in 1 large square} \times \text{Dilution factor}$$

Volume factor (0.1)

Isolated white blood cells (10x dilution factor in saline solution) were infused into the star shaped acoustofluidic device without (control) and with actuation of the device at exposure time of 350 ms. After each experiments, samples were analysed using microscopy imaging. Fig. S3. presents microscopy imaging of WBCs before and after exposure. The presence of intact white blood cells are evident for control samples (Fig. S3a). After exposure to streaming field, approximately all of the white blood cells have been destroyed (Fig. S3b). These results are similar to RBCs presented in Fig. 5. One could explain that, since WBCs are larger than RBCs, upon exposure to acoustic field the chance of lysis is higher than observed results from RBCs. Therefore, it could be suggested the device lyses both RBCs and WBCs at the same time homogenising whole blood.

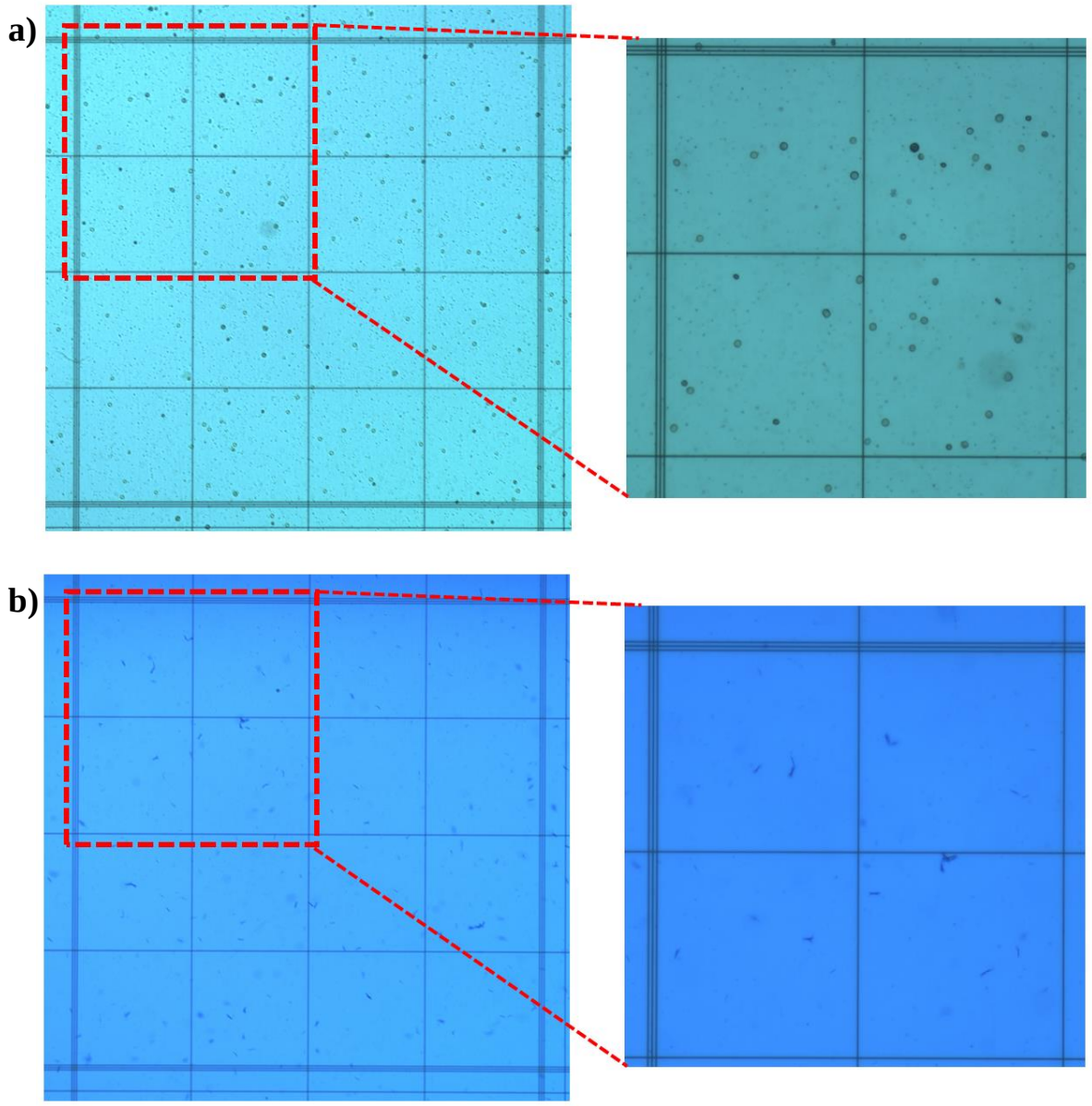

Figure S 3. Microscopy imaging of stained white blood cells (WBCs) a) before (control) and b) after 350 ms exposure.

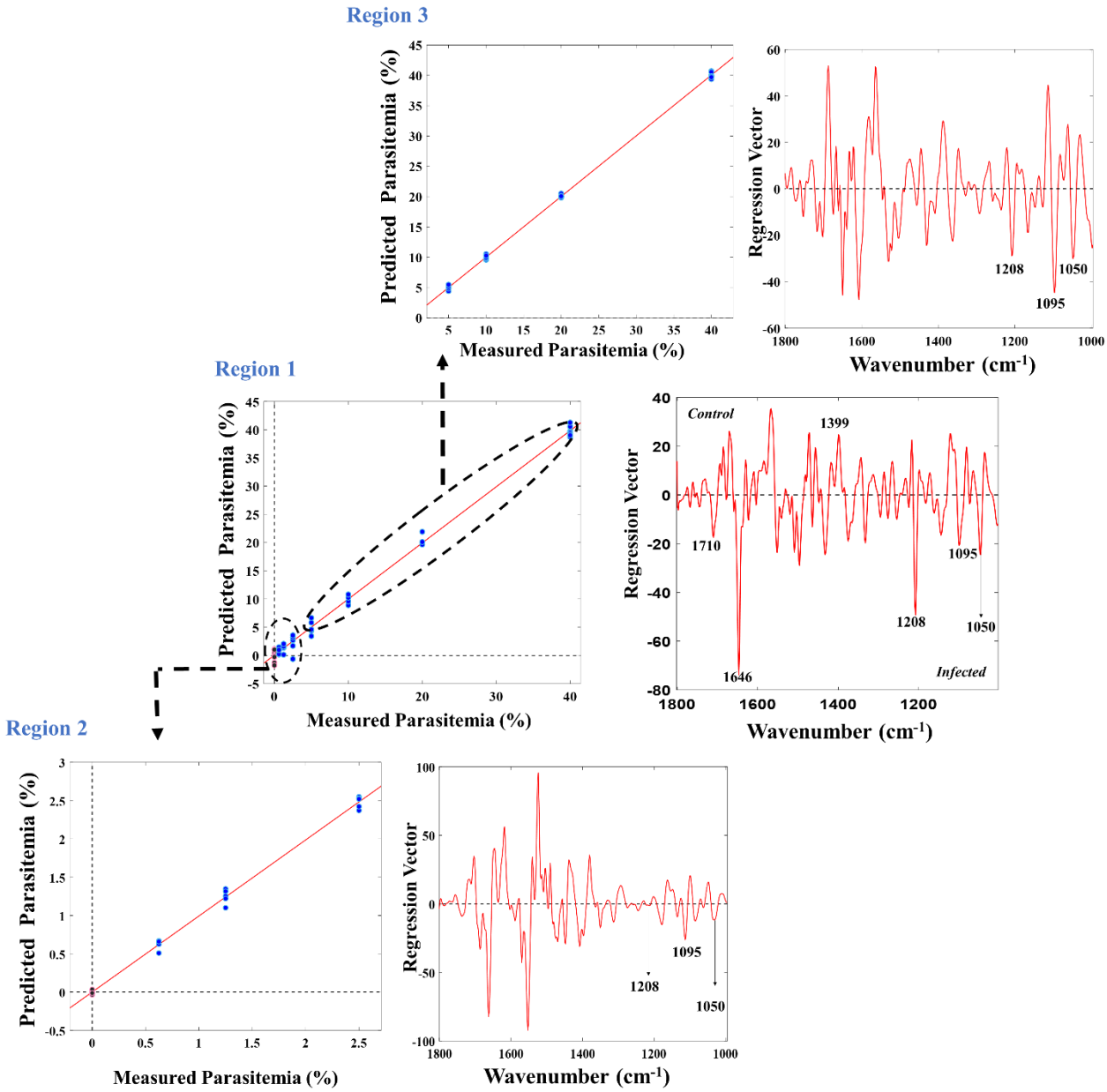

Figure S 4. PLS- regression scores plot of acoustically lysed malaria sample sets in Region 1) 0 – 40% parasitemia range, Region 2) 0 – 2.5% parasitemia, and Region 3) 5 – 40% parasitemia range. The corresponding regression vectors are shown along with its scores plots.

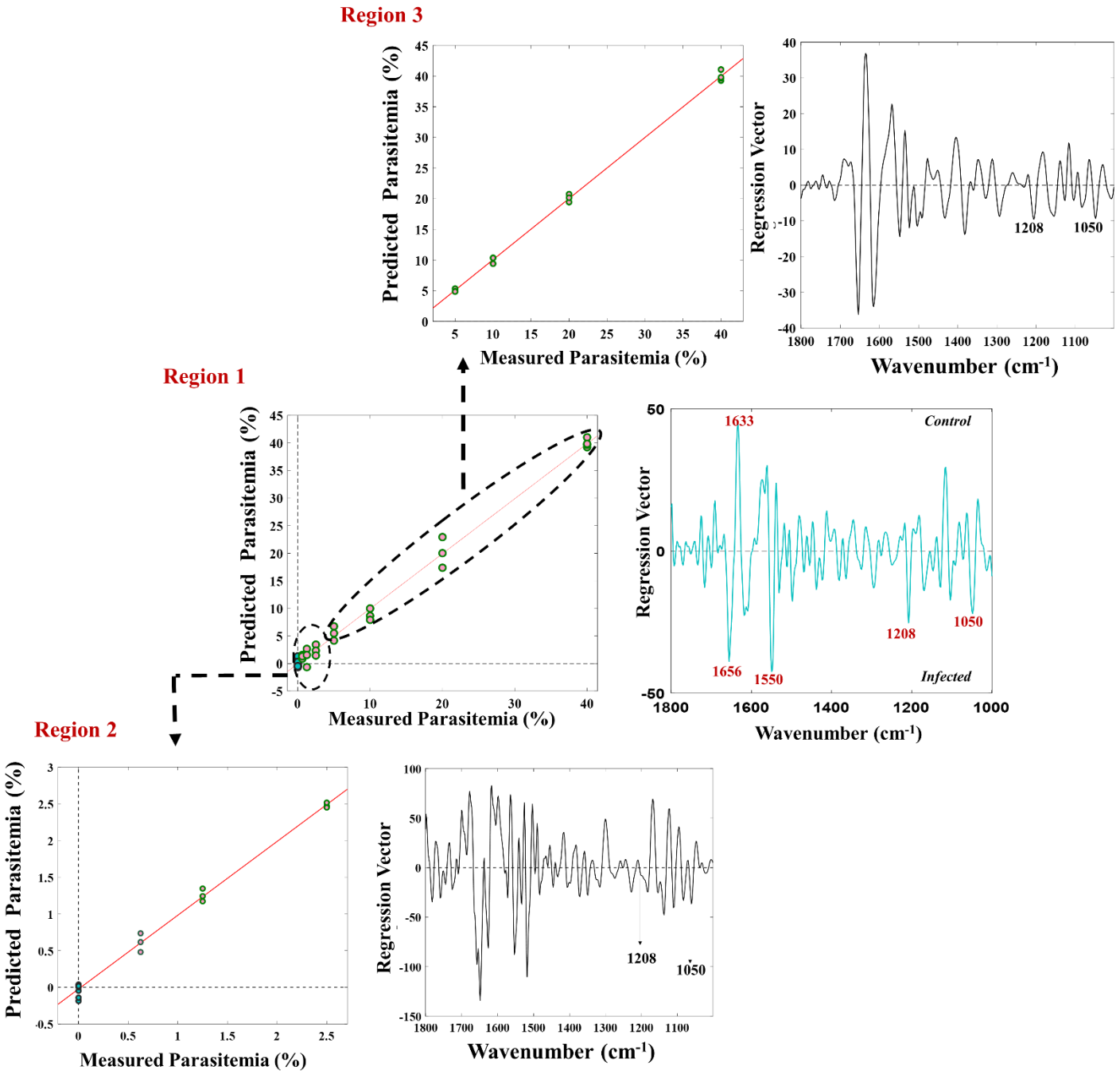

Figure S 5. PLS- regression scores plot of non- lysed malaria sample sets in Region 1) 0 – 40% parasitemia range, Region 2) 0 – 2.5% parasitemia, and Region 3) 5 – 40% parasitemia range. The corresponding regression vectors are shown along with its scores plots.