Supplementary information

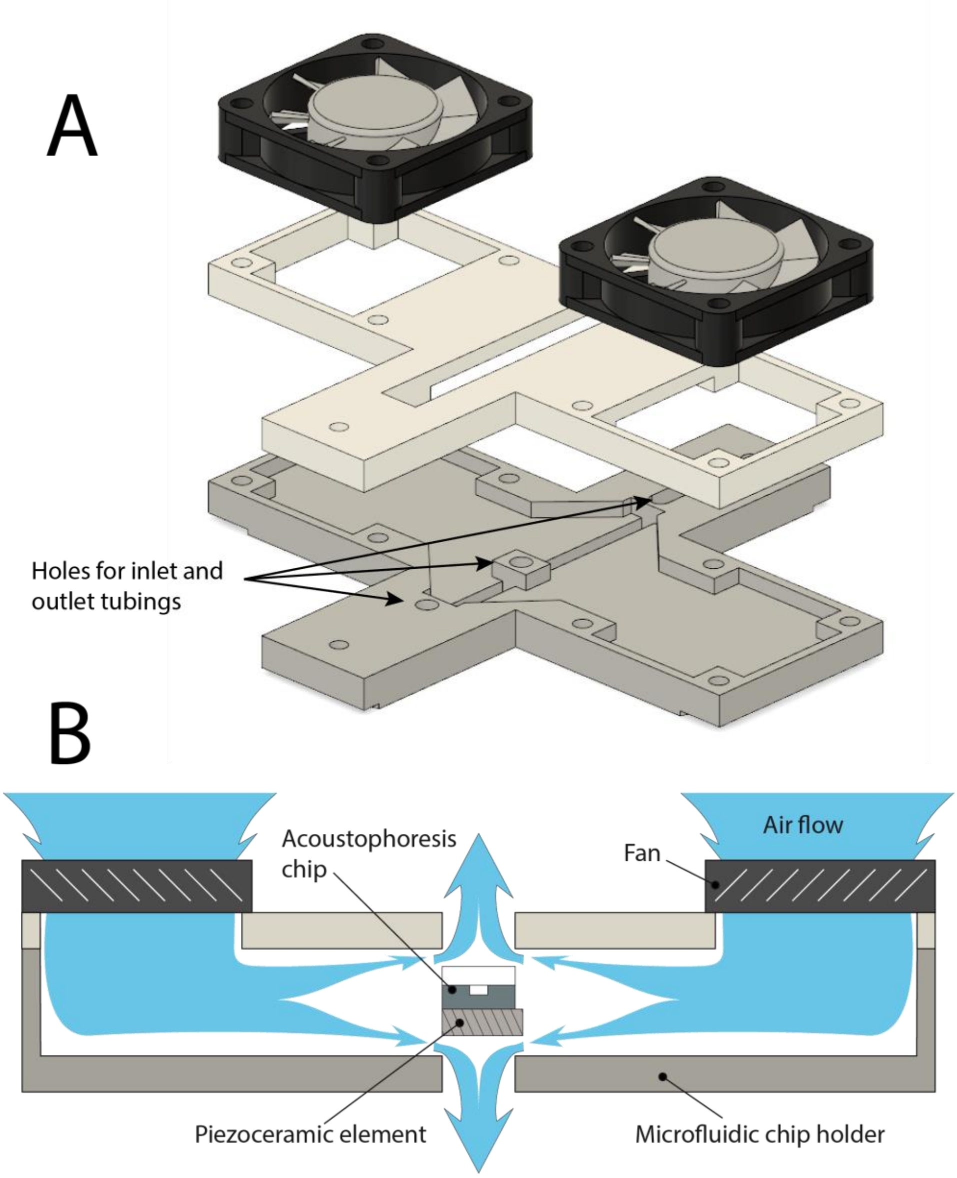

Figure S1. A) Exploded view of the microchip holder with air cooling. Dark grey bottom layer, white top layer and the two axial fans (black). B) Schematic cross section of the air flow inside the air-cooling manifold.

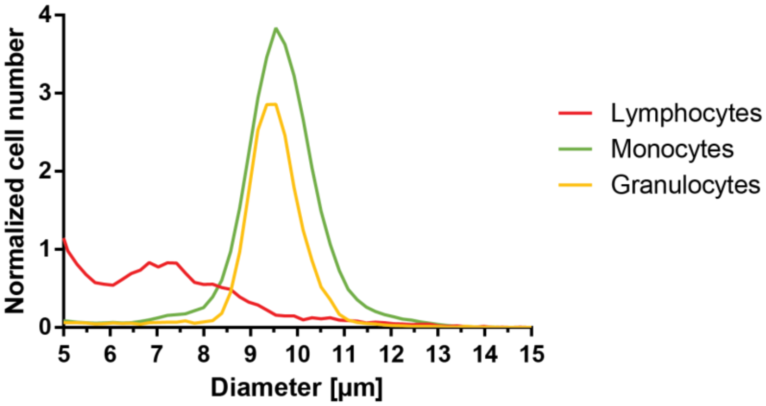

Figure S2. White blood cells were acoustically fractionated into lymphocytes (red), monocytes (green) and granulocytes (yellow) with purities >90% and cell sizes were determined using a Multisizer 3 Coulter Counter. Note, for the lymphocyte population the background of dead cell debris is relatively high leading to an increased signal in the diameter range of 5-6µm.

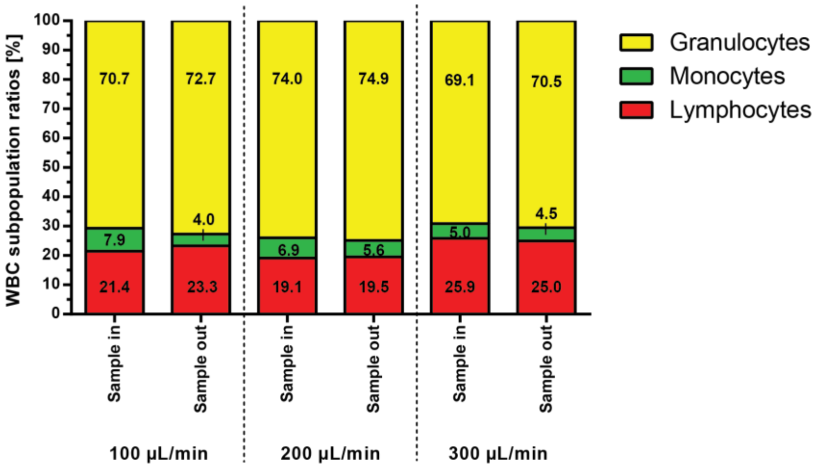

Figure S3. Ratio of WBC subpopulations before and after acoustic separation are shown at different sample flow rates. At low sample flow rate there is a discrepancy in the WBC subpopulation ratio. This is due to the considerably lower flow rate in the side1 outlet, i.e. monocyte outlet, and the dead volume in the sample tubing. By running larger sample volumes or by flushing the tubing's after the acoustic run the remaining cells can be recovered. The effect is less prominent at higher sample flow rates.

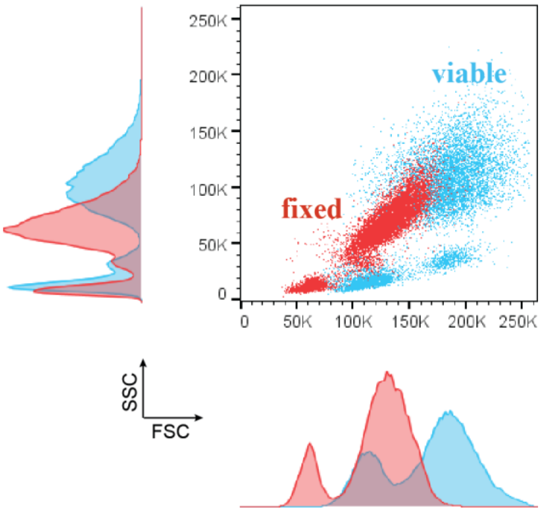

Figure S4. FACS plot with forward scatter (FSC) and side scatter (SSC) signal are shown for fixed (red) and viable (blue) white blood cells indicating a shift in the relative size and granularity of the WBC subpopulations due to fixation of the cells.

$$F_z^{rad} = \frac{4}{3}\pi\phi(\tilde{\kappa},\tilde{\rho})ka^3E_{ac}\sin(2kz)$$

$$E_{ac} = \frac{p_a^2}{4\rho_0c_0^2}\,;\;\; \phi(\tilde{\kappa},\tilde{\rho}) = \frac{5\tilde{\rho}-2}{2\tilde{\rho}+1} - \tilde{\kappa};\qquad \tilde{\kappa} = \frac{\kappa_p}{\kappa_0};\qquad \tilde{\rho} = \frac{\rho_p}{\rho_0}$$

Supplementary Equation 1. Acoustic radiation force F_z^{rad} acting on a particle with the radius ɑ in an acoustic standing wave field where κ_0, ρ_0, κ_p and ρ_p are the compressibility and density of the fluid and particle, $\phi(\tilde{\kappa},\tilde{\rho})$ is the acoustic contrast factor, k is the wave number (2π/λ), E_{ac} is the acoustic energy density, z is the position of the particle along the wave propagation axis, p_a is the pressure amplitude, c_0 is the speed of sound in the medium[28].

$$Acoustophoretic\ mobility = \frac{a^2\phi}{\eta}$$

Supplementary Equation 2. Acoustophoretic mobility of a particle in an acoustic standing wave field where a is the radius and ϕ the acoustic contrast factor of the partice and η is the viscosity of the medium.