Supplementary Material (ESI) for Lab on a Chip

Support Information

Self-Contained, Self-Powered Integrated Microfluidic Blood Analysis System (SIMBAS)

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Fabrication of SIMBAS

The microfluidic channels were fabricated using standard soft lithography replica molding techniques[13]. Briefly, a mould was created through a single-layer process using negative photoresist, SU8-2100 (Microchem U.S.A.), which was spun onto a clean silicon wafer using a spin-coater (P6700 Specialty Coating Systems, Inc., U.S.A.) to form an 80-µm thick layer. The photoresist was poured onto the wafer at 500 rpm; the angular speed was then ramped up to 2500 rpm for 30 sec with an acceleration of 300 rpm/s. Next, the wafer was soft-baked at 65 ºC for 5 min and 95 ºC for 30 min, followed by UV-exposure for 10 s at 9.5 mW/cm^2 using a mask aligner (Karl-Süss KSM MJB-55W). The wafer was then baked for 5 min at 65 ºC and 12 min at 95 ºC, and allowed to cool to room temperature. Finally, the wafer was developed in SU8 developer (Microposit EC Solvent, Chestech Ltd., UK) for 4 min, rinsed with isopropanol, and blown dry using N_2.

PDMS (Sylgard 184, Dow Corning) was prepared with a 10:1 mass ratio (base to cross-linker); degassed in a vacuum chamber for 30 min; then poured on the SU8 mold to a thickness of ~ 2mm; then cured in an oven at 60 ºC for at least 10 h. The PDMS was then carefully peeled off the mould. The PDMS was punched with a 2-mm outer-diameter flat-tip needle (Technical Innovations, Inc, Texas, USA) to form the circular filter trenches. Up to 5 trenches were punched in one chip. The PDMS fluidic layer was placed in conformal contact with the glass slides, providing reversible sealing.

Considering that the microfluidic mould is produce once and is sufficient for making multiple chips, the production of a single SIMBAS chip requires 4 steps:

1. Casting and curing the PDMS on the microfluidic mould.
2. Peeling, and punching the filter trenches.
3. Protein pattering of the bio-recognition site on the upper glass slide.
4. Placing the PDMS fluidic layer in conformal contact with the upper and lower glass slides.

Fabrication of PDMS stamps for microcontact printing

Prior to assembling the device, the bio-recognition site on the top glass slide was patterned by microcontact printing[13] to create 15-µm-wide stripes of avidin (Sigma Aldrich, U.S.A.) Patterned PDMS stamps were fabricated by pouring a 10:1 (v/v) mixture of Sylgard 184 elastomer and curing agent over a patterned silicon master. Fabrication of the patterned silicon master was done as follows: MICROPOSIT™ S1818™ Positive Photoresist was spun at 5500 rpm for 30 sec on a silicon wafer. The coated wafer was then cured for 1 min on a vacuum hot plate at 115 °C. UV light irradiated the photoresist layer for 20 sec through a photomask (Photronics, Mid Glamorgan, South Wales, UK). Resultant features were developed by dipping the master in developer MF319 (Chestech Ltd, Warwickshire, UK) for 40 sec; finally, it was rinsed with water and dried under nitrogen. Subsequently, masters were exposed to a vapor of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (Sigma Aldrich Inc., Ireland) under vacuum for 1 h to facilitate the release of the PDMS mold after curing.

The mixture was cured for one hour in an oven at 60 ºC, then carefully peeled away from the master and left in the oven for another 18 h at 60 ºC to ensure complete curing. Prior to inking, the stamps were oxidized by exposure to UV/ozone for 10 min. This process causes the stamp surface to become hydrophilic, which ensures homogeneous spreading of the ink (*i.e.*, the protein solution). The stamps were freshly prepared no more than two days prior to use.

Protein-Patterned Surfaces

Standard microscope slides were used as glass substrates for the protein patterning. The slides were rinsed with ethanol and N_2 dried. The PDMS stamps were ozone-activated and immediately inked with 50 µL of a 200 µg/mL Neutravidin (a deglycosylated version of avidin, Sigma Aldrich, U.S.A) solution in phosphate-buffered saline (PBS) for at least 15 min. Excess ink solution was removed from the PDMS surface with a pipette and the stamp was then blown dry with nitrogen. The stamp was brought into contact with the glass substrate for 5 min. After protein micropatterning, the glass slides were immersed in BSA solution (10 mg/mL) in PBS, blocking any uncovered regions of the glass surface, for at least 1 h at room

temperature. The slides were rinsed with PBS and water, and then N_2 dried prior to assembling the device and using it for blood analysis. Typically, patterned substrates were used on the same day as their preparation.

Flow rate characterisation

Flow rates were characterised by loading the device with 10 μM 7-dichlorofluorescein (DCF) green fluorescent dye dissolved in Milipore pure water (Millipore, U.S.A.). The loaded device was placed on an IX80 (Olympus, Japan) inverted fluorescence microscope and irradiated for 100 ms every 40 sec with a 492-nm excitation beam (excitation filter BP492/18 with a xenon light source, CellR MT20, Olympus); 530-nm fluorescent light was sampled through a filter cube (U-MF2, Olympus) with a CCD sensor (Hamamatsu C4742-80-12AG). The images were analysed with the CellR software package to reveal the kinetics of the channel filling.

Simulations

The flow patterns were calculated by means of the two-dimensional Navier-Stokes equations for total continuity, energy, and momentum.

$$0 = \nabla u \tag{1}$$

$$\rho \frac{\partial u}{\partial t} + \rho u \cdot \nabla u = \nabla \cdot [-\rho I + \eta \nabla u] + F \tag{2}$$

where u is the velocity of the flowing mixture, ρ is the fluid density, I is the inertia force, μ is the dynamic viscosity, and F is the external body force.

To estimate blood cell (*i.e.* particle) trapping efficiency in the micro-trench system, the Kahn and Richardson force for particle trajectory was calculated. In order to accurately explain the process dynamics, this work focused on the effects of the geometrical parameters, flow velocity, and particle trapping efficiency (see Table 1). The pressure boundary condition was used for the inlet and the outlet. In the computational analysis, 50 particles were tracked, and estimated boundary conditions were used, as listed in Table 2. The trench simulation was performed in a two-dimensional triangular grid consisting of 1,168 cells using commercial computational fluid dynamics (CFD) software, COMSOL ver 3.4 and CFD-ACE. The semi-implicit pressure-linked equation (SIMPLE) algorithm was applied to solve the momentum equation. The calculation for each case took about 5 min of run time on an Intel Xeon E5420 @ 2.50 GHz.

Table 1. Flow and device parameters

Parameter	Values
Relative* trench height h_1/h_0	0.25, 2.5, 6.25, 12.5, 25
Relative* trench length L/h_0	0.25, 2.5, 6.25, 12.5, 25
Fluid and particle velocity (m/s)	9.0×10^{-2}, 1.8×10^{-3}, 9.0×10^{-3}, 9.0×10^{-4}, 9.0×10^{-5}
Particle diameter, D_p/h_0	2.5×10^{-1}
Particle density (kg/m^3)	1.1×10^3

*relative to inlet channel height, h_0

Table 2. Boundary conditions

Boundary	Condition	Value
Inlet	Pressure boundary, P_{in}	0.04, 0.4, 4, 20, 40 Pa
Outlet	Pressure boundary, P_{out}	0
Wall	No slip	-

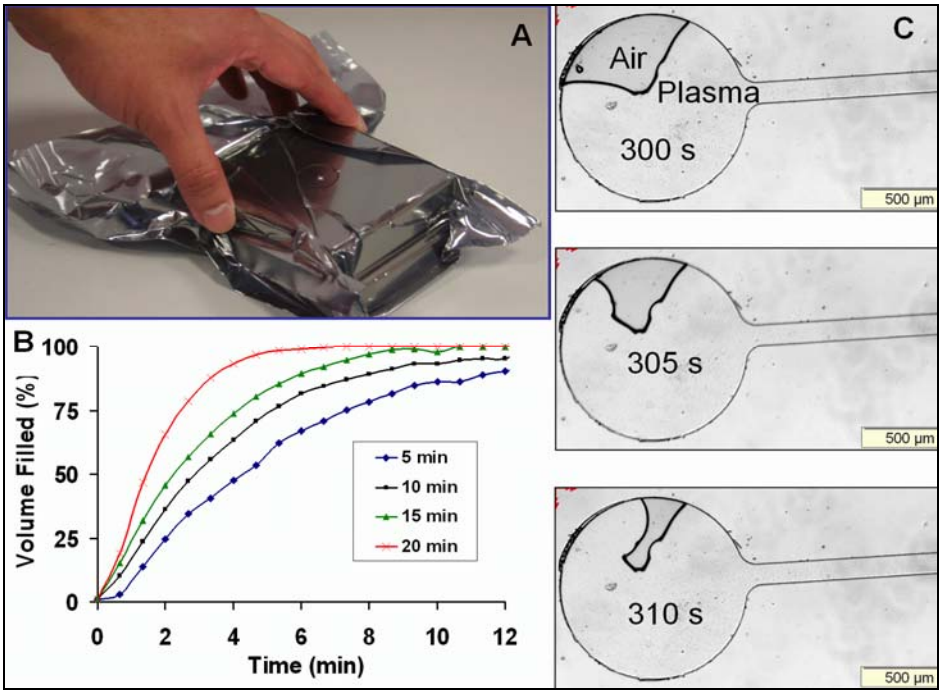

Fig. S1 (A) Degas-driven flow is generated when the SIMBAS device is removed from a low-pressure environment and a fluid sample introduced. (B) Different degassing times t_d were tested (5 min to 20 min) for generating degas-driven flow into dead-end channels made of PDMS. (C) Monitoring the filling process after degassing the PDMS device in a standard vacuum desiccator.

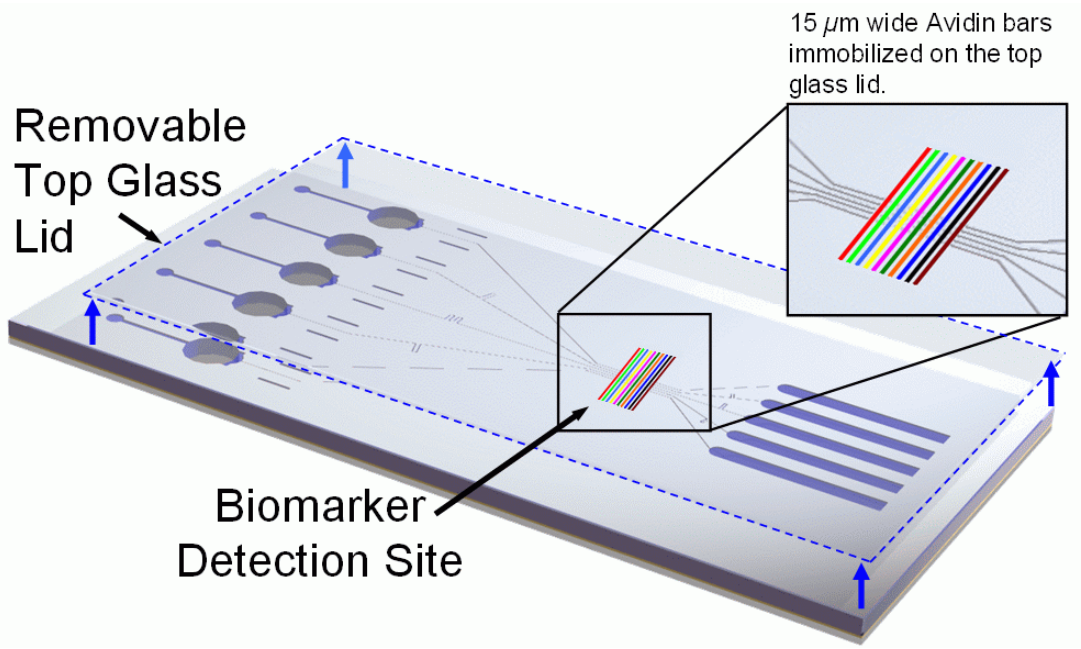

Fig. S2 Analytes from the biomarker detection matrix can be accessed for further analysis such as PCR, CE, MS, etc. by disassembling the chips after the assay: only reversible PDMS-glass bonding is used.

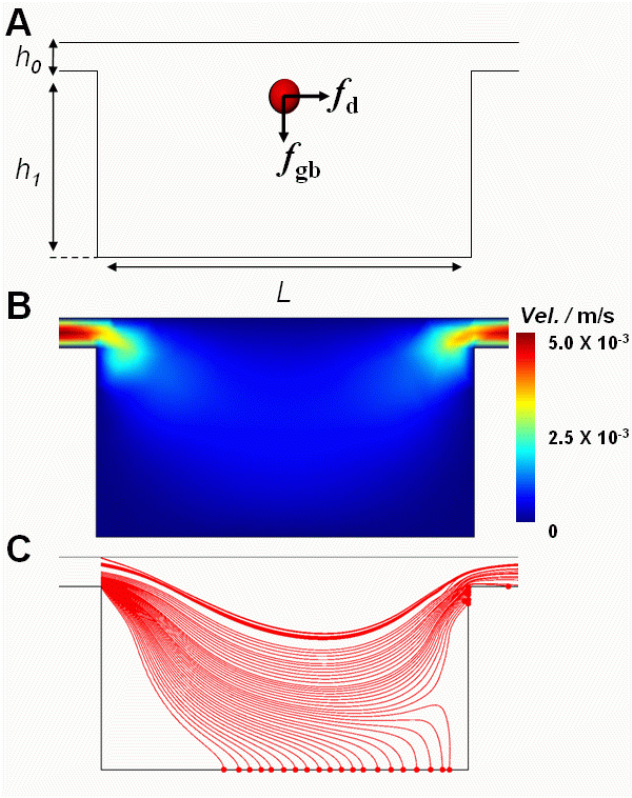

Fig. S3 Modelling and simulation of particle capture by the SIMBAS microfluidic trench system. (A) The principal forces acting on a blood cell or suspended particle within the trench are the buoyancy-corrected gravitational sedimentation force f_{gb} and the fluid drag force f_d. (B) Fluid velocity field within the microfluidic trench system as calculated by the two-dimensional Navier-Stokes equations for total continuity, energy, and momentum. (C) Particle trajectory traces for multiple particles with variable initial positions. Most particles are captured or filtered out, but a few of those that start near the top of the inlet channel escape to the outlet.