Integrated Microfluidic Array Plate (iMAP) for Cellular and Molecular Analysis Ivan K. Dimov, Gregor Kijanka, Younggeun Park, Jens Ducrée, Taewook Kang and Luke P. Lee Support Information

Fabrication:



Figure S1: The fabrication process of iMAP has been optimized to simplify alignment and complexity. It consists of 2 layers of PDMS (a fluidic layer and a lid/inlet layer) and support glass layer.

Bacterial Cell Capture:

E. coli bacterial cells have also been successfully captured within the processing camber trench. Firstly the *E. coli* solution is introduced into the device, as shown in figure S2.



Figure S2: GFP *E. coli* were captured with the processing chamber allowing bacterial cell based assays to also be done with the iMAP. (A) Initially the device is loaded with

the bacterial solution, (B) After which a washing solution is loaded in order to rinse out any non captured bacteria. Due to the low flow field at the bottom of the processing chamber trench, the bacteria present there will be effectively captured and not washed away. Due to the very low density of the *E. coli* bacteria, the capture efficiency is much lower than that of denser particles or cells such as cancer cells.

Low Volume Fluid Loading with an Oil Layer:

In cases where reagent or sample volumes are very limited and scarce an oil layer may be used to hydrostatically drive in the low volume reagents. Experiments with 20 microliter tips demonstrated that volumes as low 400 nL can be readily loaded into the processing module. Since each module consists of 8 processing chambers each chamber is loaded with 50 nL. Due to the oils lower density, input flow rates can be upto 25% slower compared to water solution based hydrostatic flow.



Figure S3: Reagent volumes as low as 50 nL can be loaded into the processing chamber by using a mineral oil layer. This reduces the reagents costs and allows the device to be used in applications where sample or reagent volumes are extremely limited.

Cell Capture Efficiency:



Figure S4: (A-B) CFD simulation results of the flow velocity magnitudes in the processing chamber and trench. Cell capture is achieved due to the flow velocity magnitude in the trench being approximately 3 orders of magnitude lower the flow above it. So particles that enter the low flow velocity region are effectively captured. Experiments with 1 μ m silica beads (C) demonstrate the capture efficiency of the structure. See support movie SM2 (D-E) cancer cells (HeLa) are also efficiently captured in the processing chamber.



Figure S5: Protocol with the main steps involved in an integrated cell stimulation assay with gene and protein expression analysis.



Figure S6: With on-chip gravity driven flow control, iMAP is flexible and can be easily integrated into existing infrastructure and workflows such as robotic pipetting systems (A), incubators (B), and fluorescent microscope systems (C).



Figure S7: Photograph of the complete iMAP, which has nanolitre-well array plate for cell-based assay. The plate is designed for versatility that allows the user to seamlessly combine any set of steps required in a cell based or particle based assay.



Figure S8: Fluid loading and mixing unit operation is achieved due to the slow diffusion mixing of the input fluid with the previous contents of trench. (A) Experiments with fluorescent dye (DCF) mixing with water. (B) Dependence of the mixing speed on the input flow velocity. (C) Simulations demonstration the mixing of DCF with water within the trench. The fluid loading and mixing unit operation enables the execution of some basic cell based assay procedures such as cell culture and staining (D) and cell lysis (E), a bright field time lapse of that process can be seen in the support movie SM5.

Computational Fluid Dynamics:

The flow patterns are calculated by means of two-dimensional Navier-Stokes equations of the 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 mixture flow, ρ is the flow density, *I* is the inertia force, μ is the dynamic viscosity, and *F* is the external body force.

To estimate particle trapping efficiency in this trench system, Kahn and Richardson force for particle tracing is calculated.

$$F = \pi r_p^2 \rho (\bar{u} - \bar{u}_p)^2 (1.84 (\text{Re}_p)^{-0.31} + 0.239 (\text{Re}_p)^{0.06})^{3.45}$$
(3)

where the definition of the particle Reynolds number, Re_{ρ} , is

$$\operatorname{Re}_{p} = \left(\left| \overline{u} - \overline{u}_{p} \right| 2r_{p} \rho \right) / \eta$$
(4)

The orientation of a given force component (for example, positive or negative *x*-component) is determined by the sign of the corresponding component in the vector difference, because this determines whether the fluid is accelerating or slowing down the particle in that direction.

Mass transfer and component mixing are estimated from the mass transfer equation.

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D\nabla c + c\mathbf{u}) = 0$$

where, c denotes the concentration, D is its diffusion coefficient, and **u** refers to the velocity.

(5)

This process was designed to trap particles from the mixture. In this simulation, the particle trap and mixing effect were the key factors for predicting the real process. In order to accurately explain the process dynamics this work focused on the effects of the geometrical parameters, flow velocity and particle size, particle trapping efficiency and the mixing of fluid components (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 as listed in Table 2 were used. The trench simulation was performed in a two-dimensional tri-angular grid consisting of 1,168 cells for estimation of trapping efficiency and 4,905 cells for mixing effects using a commercial computational fluid dynamics (CFD) code, COMSOL ver 3.4 and CFD-ACE, respectively. 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 running time on a Intel Xeon E5420 @ 2.50 GHz.

Tn is the time to complete species replacement within the trench. It is defined as the time that is takes for a new added species to reach a stable final concentration in the trench and is calculated as the difference in the time between the added species normalized average concentration of 1×10^{-4} and 0.99 at a given constant flow rate of the added species. Thus *Tn* depends amongst other things on the flow velocity of the added species, the diffusion coefficient of the added species, the trench geometry and the inlet and out flow path lengths.

Table 1. Boundary condition	
Parameter	Values
Trench height	0.25, 2.5, 6.25, 12.5, 25
Trench length	0.25, 2.5, 6.25, 12.5, 25
Velocity	$9.0 \times 10^{-2}, 1.8 \times 10^{-3}, 9.0 \times 10^{-3}, 9.0 \times 10^{-4}, 9.0 \times 10^{-5}$
Particle diameter, D_p/H_0 at D =	2.5×10^{-3} , 7.25×10^{-3} , 2.5×10^{-2} , 7.25×10^{-2} ,
1.9× 10 ⁵ ,	2.5×10^{-1} ,
Particle density, D	1.9×10^2 , 1.9×10^3 , 1.9×10^4 , 1.9×10^5 , 1.9×10^6 ,

Table 2. Boundary	condition	
Boundary	Condition	Value
Inlet	Pressure boundary, <i>P_{in}</i>	0.04, 0.4, 4, 20, 40 Pa
Outlet	Pressure boundary, Pout	0
Wall	No slip	-



Figure S9: Particle effect on the capture efficiency η . (A) Diagram defining the simulation parameters (B) Velocity field calculation and particle trajectory trace for a single parameter combination. Unless other wise shown the geometry parameters are kept constant at $h_1/h_0 = 6.25$, $L/h_0 = 2.5$ (C) Effect of the particle size on the capture efficiency (D) Effect of the particle size on the capture efficiency.

.



Figure S10: Mixtures can be maintained within the trench for long periods of time without the need for active valves. If the flow is stopped at a time ts < Tn then a mixture is created and maintained as shown in the figure. For variation of $\pm 10\%$ the mixture can be stable for over 150 min. Simulation parameters: diffusion coefficient $D=5.9 \times 10^{-9} \text{ m}^2/\text{s}$ geometry $h_1/h_0 = 6.25$, $L/h_0 = 2.5$. For proteins or bigger biomolecules, D is usually 2 orders of magnitude smaller ($D \sim 10^{-11} \text{ m}^2/\text{s}$) so mixtures are more and last for longer times.

Supporting Information Movies:

SM1. *iMAP* processing module priming based on the degassing method. Movie file: <u>SM1.priming.bubble.removal.avi</u>

SM2. *iMAP* 1 micro particle capture with very high efficiency (**3 movies**). Movie file: <u>SM2a.1um.silica.bead.capture.avi</u> Movie file: <u>SM2b.1um.silica.bead.capture.avi</u> Movie file: <u>SM2c.1um.silica.bead.capture.avi</u>

SM3. *iMAP* GFP *E. coli* loading. Movie file: <u>SM3.GFP.EColi.loading.avi</u>

SM4. *iMAP* captured GFP *E. coli* with washing flow above them. Movie file: <u>SM4.Captured.GFP.EColi.during.washing.step.avi</u>

SM5. Lysis movie. Movie file: <u>SM5.Lysis.HeLa.cells.avi</u>

SM6. Real Time NASBA movie showing the amplification of 2x10⁶ *E. coli*/mL lysate. One frame is captured every minutes for 90 min. Movie file: <u>SM6.Real-time.NASBA.avi</u>

SM7. Mixing simulation. Movie file: <u>SM7.Mixing.Simulation.mpg</u>

SM8. Cell loading movie of plasma cells U266 with an inlet velocity of ~300 µm/sec. Movie file: <u>SM8.Cell.loading.movie.avi</u>