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

Static droplet array for culturing single live adherent cells in an isolated chemical microenvironment

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Fig S1:

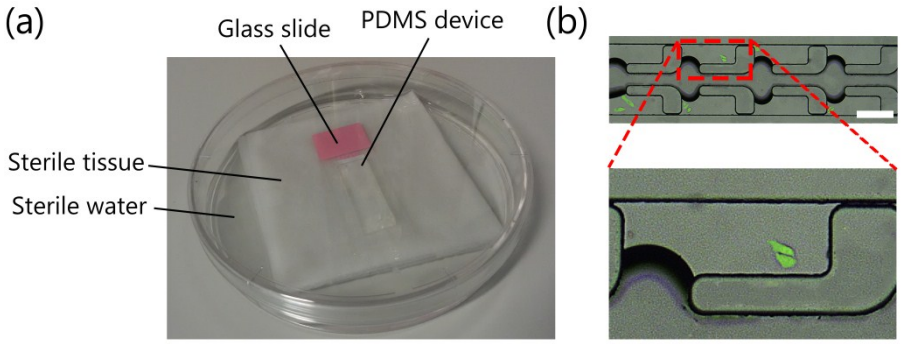

Figure S1: Cell culture in the device: (a) Device setup by creating a humidity chamber (b) Cell attachment to the substrate; here two cells are located just adjacent to each other, Scale bar=200µm in all images.

Fig S2:

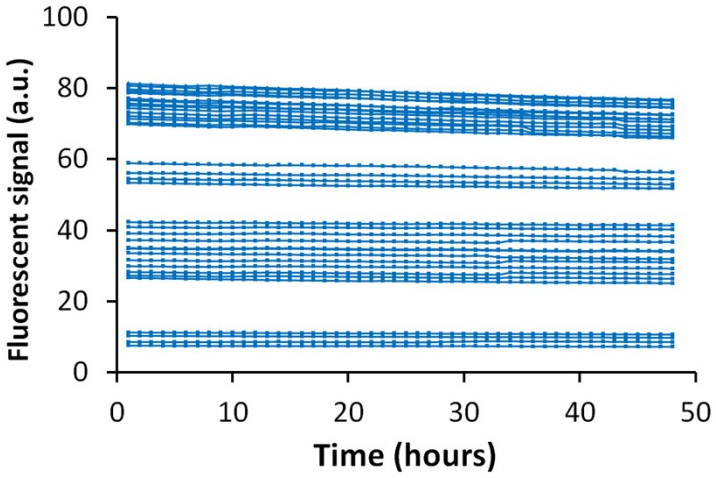

Figure S2: Chemical isolation of the droplets throughout the experiment. The device is filled with different concentrations of fluorescein and the fluorescent intensity of droplet was measured over 48 hours experimental period. Each line belongs to a unique droplet.

Fig S3:

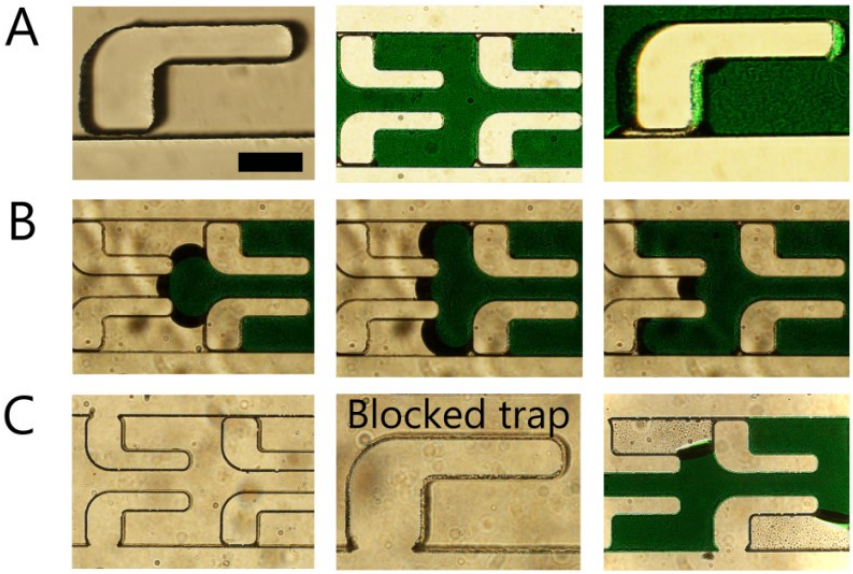

Figure S3: A) The geometry of the narrow restriction, B) image sequence showing the liquid-air interface movement in the trap during the sample injection, C) liquid-air interface when moving towards a defective trap with blocked restriction at its end (Scale bar=100µm).

Fig S4:

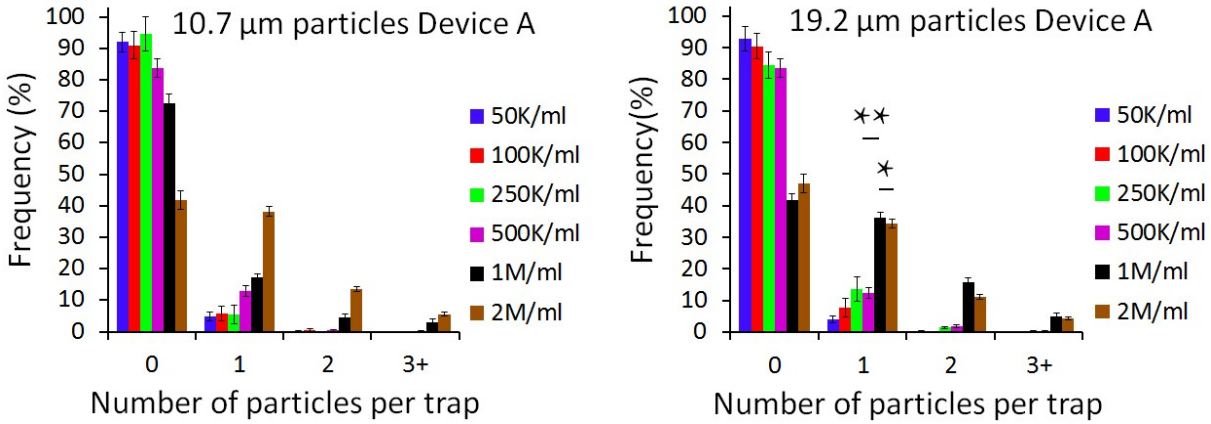

Figure S4: Device characterisation: (A) Trapping different concentration of particles inside droplets with regards to the solution concentration and particle size in static droplet arrays (n=9, (*): p<0.05; (**): p<0.01); N=500 droplets

Fig S5: single cell trapping efficiency versus flow rate

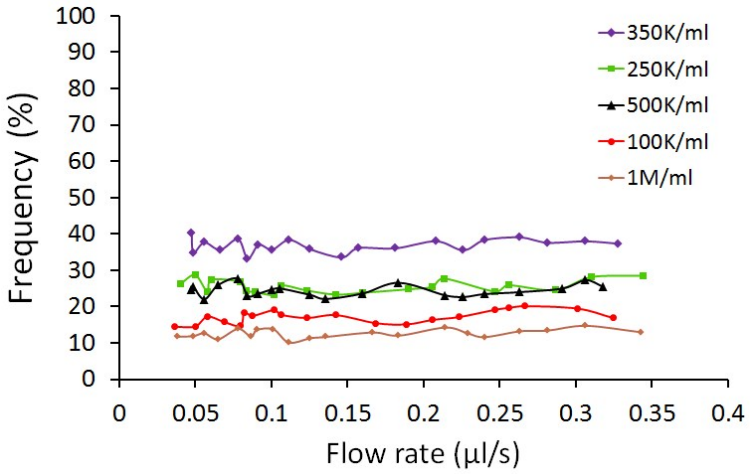

Figure S5: single cell trapping efficiency versus flow rate

Fig S6:

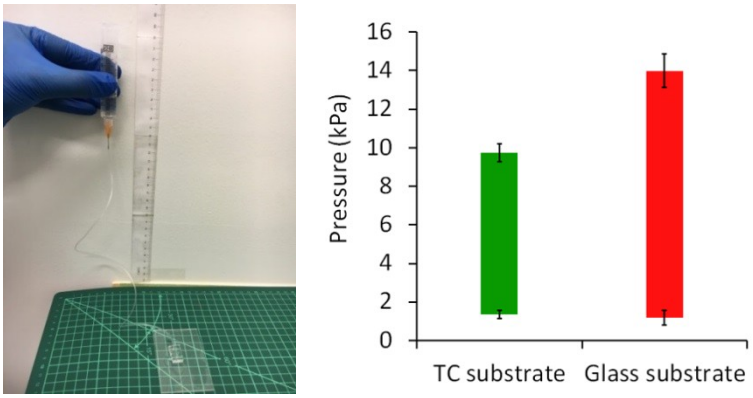

Figure S6: Pressure test experimental set up; The pressure range applied at the inlet to drive the liquid into the traps during loading step with no leakage (n=5).

A sample of pressure limit calculation is provided below:

For the TC substrate,

h_{low} =0.138 m → P_{low}= ρgh= 998.23×9.8×0.138= 1350.006 Pa= 1.350 kPa

h_{up}=0.994 m → P_{up}= ρgh= 998.23×9.8×0.997= 9753.306 Pa= 9.753 kPa

Fig S7:

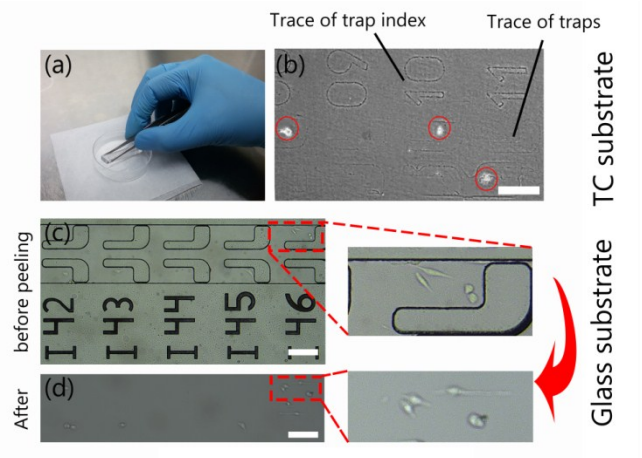

Figure S7: Device peel-off: (a) Peeling step using a pair of tweezers. (b) Cell retrieval following peeling from TC-substrate; the trace of traps as well as trap indices are clearly visible enabling cell retrieval and cell addressability with high accuracy. (c) Successful peeling of the device from a glass substrate, Scale bar=200µm in all images.

Fig S8:

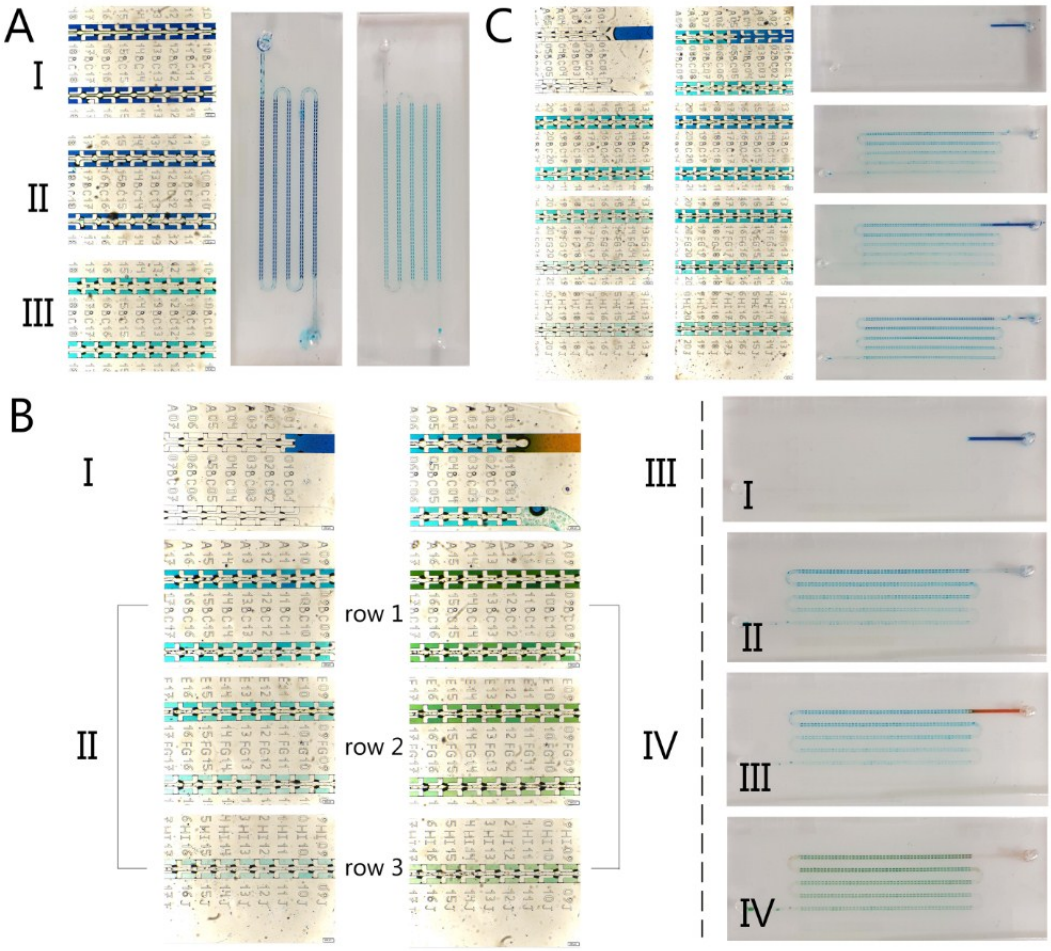

Figure S8: Multistep dilution and gradient generation in static droplet arrays A) Multistep dilution B) Two-colour gradient generation with two step addition of a 0.187 µl plug of blue and red food colour respectively. C) Gradient generation with two step addition of a 0.187 µl plug of blue food colour. (Note: This volume is calculated as the amount of sample filling the channel from the inlet up to the first trap).

Fig S9:

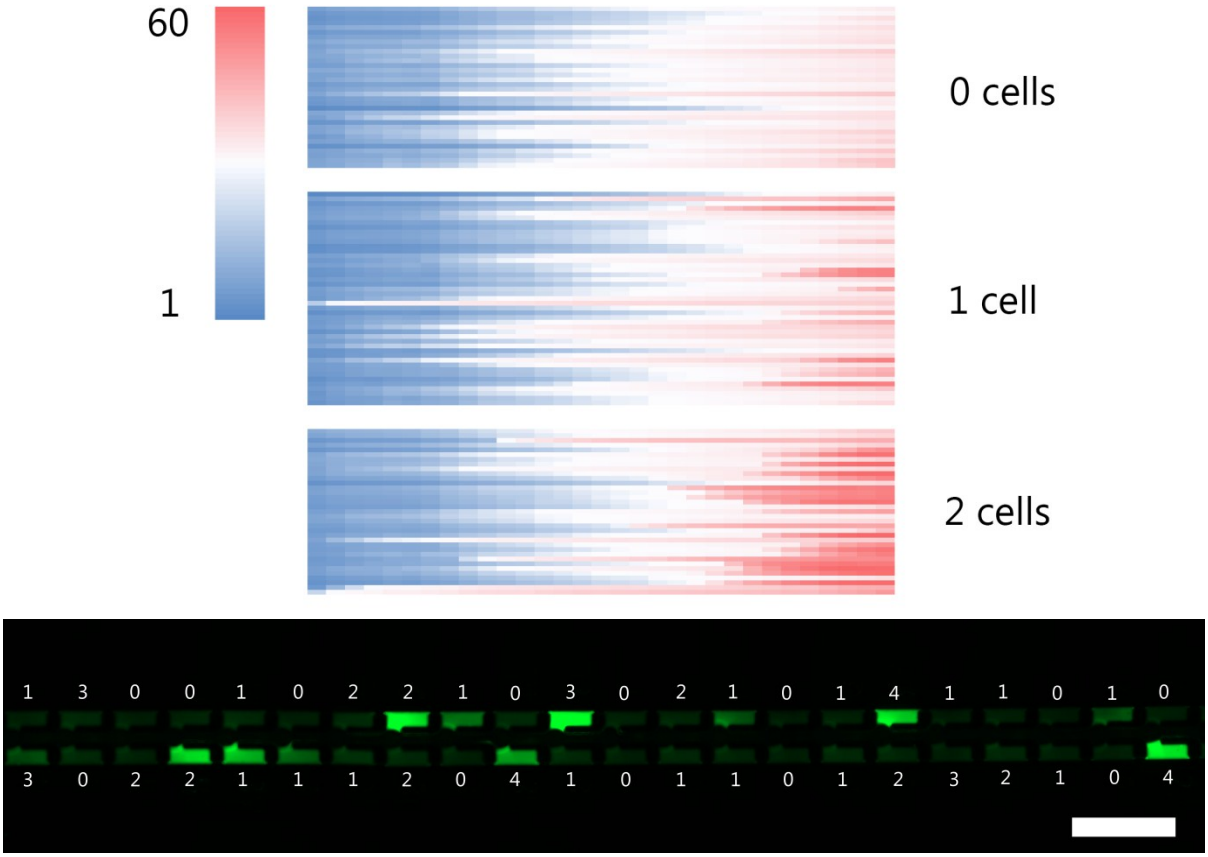

Figure S9: Cellular heterogeneity and dynamic response; heat map presenting the relative fluorescent intensity in droplets containing no cell, 1 cell and 2 cells (n=34, 45, and 35 respectively). Red color represents high MMP activity and blue color shows low MMP activity. Bottom: Image of 44 droplets containing different number of cells, Scale bar: 800 µm.

Method S1: λ Calculation

Calculation for cell concentration of C=1×10^6/ml.

$$V_{\text{single cell}} = \frac{4}{3}\pi r^3 = \frac{1}{6}\pi D^3 = \frac{1}{6}\pi(15)^3 = 1.76 \times 10^3\ \mu m^3$$

For the above cell concentration (i.e. 1×10^6 cells): $V_{cells} = 1.76 \times 10^9\ \mu m^3$

V_{media} = 1ml = $10^{12}\ \mu m^3$

$$\emptyset_s = \frac{V_{cells}}{V_{media}} = \frac{1.76 \times 10^9\ \mu m^3}{10^{12}\ \mu m^3} = 1.76 \times 10^{-3}$$

$$\emptyset_d = \frac{V_{single\ cell}}{V_{droplet}} = \frac{1.76 \times 10^3\ \mu m^3}{110 \times 300 \times 80\ \mu m^3} = 6.69 \times 10^{-4}$$

$$\lambda = \frac{\emptyset_s}{\emptyset_d} = \frac{1.76 \times 10^{-3}}{6.69 \times 10^{-4}} = 2.64$$

Likewise, λ values were calculated for other concentrations as shown in Table S1:

Table S1: Calculated values of λ for different cell concentrations

Cell concentration	λ
5×10^4	0.132
1×10^5	0.264
2.5×10^5	0.660
3.5×10^5	0.924
5×10^5	1.320
1×10^6	2.640
2×10^6	5.280

Method S2: Pressure testing of the device

As mentioned in the paper, no plasma bonding is performed between the device and the substrate. Instead the device is pressed against the substrate. Since the device bottom is flat (by using photolithography on SOI wafer), a good bonding between the PDMS slab and the substrate was achieved which allows injection of sample into the device without leakage.

In order to find the device's operation pressure limit, we performed a new experiment using a syringe filled with liquid connected via a pipe to the device's inlet, and raised at various heights (corresponding to an applied pressure of $P=\rho gh$; where ρ is the liquid density, g is the gravitational constant and h is the height difference between the syringe and the device). Fig. S6 demonstrates the experimental set up. Before putting the tube into the device's inlet, we made sure that water is just at the tip of the tube and ready to flow into the channel. The syringe was raised from the device's level. The first pressure recorded was the pressure by which the liquid start filling the channel (P_{min}). When the syringe is raised more, at some point the device starts to leak which shows the upper pressure limit (P_{max}).

Method S3: Device peeling experiment

We sought the possibility of having access to single cells following device peel off. To demonstrate this capability, both tissue culture substrate (Corning treated culture dishes, mfr. No. CLS430166, Sigma-Aldrich) and glass substrate were investigated. Autoclaved devices were first placed on top of a substrate and pressed gently to make contact. Following cell injection and device loading, cells were let to attach over a period of 6 hours. For the TC culture substrate, the dish was filled with appropriate volume of culture medium just before reaching the maximum height of the device. Next, the device was carefully peeled off the TC dish using sterile (autoclaved) tweezers. The device area was examined under a light microscope (Leica DMi1 inverted microscope, Wetzlar, Germany) and imaged. For the glass substrate the device was first carefully peeled off using a pair of sterile tweezers and then cells were covered with fresh media and imaged.

Movie S1

Video illustrating how liquid flows and fills the channel and the traps (Scale bar: 300 μm).

Movie S2

Device loading with 10.7 μm particles and shearing using air leading to segmented droplets trapped in the droplet tweezers device (Scale bar: 300 μm).

Movie S3

Formation of air plug with 20 times and 100 times magnification (Scale bar: 200 μm and 100 μm for 20 times and 100 times respectively).

Movie S4

Blocked restriction blocks liquid movement (Scale bar: 300 μm).

Movie S5

Liquid shearing in the device (Scale bar: 200 μm).

Movie S6

Sheathing the droplets with oil (Scale bar: 200 μm).