

Electronic Supplementary Information

Materials and Methods

Design, Fabrication and Packaging

The microfluidic circuits contained two inlet channels to converge cell and ligand streams at a 25- μm -wide and 200- μm -long stream thinning elements (STE), with expansion at 120° to form a 500- μm -wide incubation channel for 20-fold amplification of the initial displacement. A single operation velocity was considered, necessitating different incubation channel lengths (e.g. 0.84 mm for a 100 ms incubation and 4.2 mm for a 500 ms incubation). With the current STE and channel expansion dimensions, the shortest incubation time is 42 ms. The incubation channels terminated with bifurcation, conjoining with two mirrored STEs, again 25- μm -wide and 200- μm -long, for reaction arrest and delivery to the outlet ports.

The microfluidic switch system was replicated in poly(dimethyl siloxane) (PDMS, Sylgard 184, Dow Corning) from a 60- μm -high SU-8 master fabricated by standard photolithographic methods. Inlet and outlet ports were produced using a 1-mm-diameter biopsy punch (Kia Medical), with plug and play interconnection involving a 2 cm length of steel tubing (from a 18G needle, Terumo Medical Corporation) interfaced with PFA tubing (OD 1/16") for connection to syringe pumps (e.g. neMESYS, Centoni GmbH). To encapsulate the microchannels a thin (<200 μm) PDMS layer was prepared on a glass coverslip support as previously described.¹ For vertical focussing a lift channel, also 60 μm in depth, was replicated in this basal PDMS layer. Both PDMS layers were plasma bonded using a 0.2 mbar oxygen atmosphere treatment for 45 s (70 W, 40 kHz (Femto, Diener Electronic, Germany)). The plasma-activated PDMS microchannels were derivatised with poly-L-lysine-g-polyethylene glycol, PLL-g-PEG (100 $\mu\text{g}/\text{mL}$), a protein and cell repellent material necessary to prevent clogging within the STEs.²

Cell Preparation and Imaging

Human epithelial carcinoma cells, HeLa S3, were purchased from DSMZ (Germany) and cultured in Dulbecco's modified Eagle media (DMEM) supplemented with 10% (v/v) foetal bovine serum (PAA Germany), and 1% (v/v) penicillin and streptomycin (Sarstedt AG & Co., Germany). Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂, and harvested as a single cell suspension using a cell scraper (Sarstedt Inc, USA). For stimulation experiments, a single cell suspension was prepared in serum-free media supplemented with 25 mM HEPES to provide a 5% CO₂ equivalent environment. Cells were stimulated with 100 ng/mL human epidermal growth factor-1 (EGF, Sigma, Germany). A $\leq 1 \times 10^6/\text{mL}$ cell suspension and serum-free media containing EGF were delivered at a respective flow ratio of 1:7 using a parallel drive syringe pump system (neMESYS, Centoni GmbH). Different devices, with different incubation channel lengths, were used for stimulating the HeLa cells for different incubation periods, followed by reaction arrest in 4% paraformaldehyde (SAV, Liquid Production, Germany) by merging the stimulated cell stream with the paraformaldehyde flow at the second STE (1:7 flow ratio). Cell, ligand and quenching reagents were maintained at 37°C using a custom thermoelectric Peltier control system (Newport Corporation, Irvine, USA) Negative controls were combined, on chip, with serum-free

media. Positive controls were treated with 100 ng/mL EGF in a Petri dish for 5 minutes, followed by reaction arrest with paraformaldehyde.

An inverted microscope (IX71, Olympus, Germany) was used to film cell transport through the microfluidic switch system. Rapid (~100 mm/s) cell transport through the STE was recorded using a high-speed camera MotionBLITZ EoSens[®] mini2 (Mikrotron GmbH, Germany) with object triggering for 10,000 frames per second capture (10 μ s exposure). For immunofluorescent staining, ~25 μ L droplets containing cells were directly collected onto a poly-L-lysine-coated glass coverslip from the outlet tubing. These were incubated at 37°C for 10 minutes. The electrostatically-tethered cells were then washed once with 200 μ L of cold (4°C) Staining Buffer (Becton Dickinson). The cell samples were then cooled in pre-chilled (-20°C) Permeabilization Buffer (Perm III, BD) and incubated on ice for 90 minutes. Samples were then washed twice with Staining Buffer (BD) at room temperature. Samples were incubated overnight at 4°C with rabbit IgG primary antibody for pY1173 (Cell Signaling, USA) followed by five washes in Staining Buffer. A Cy3-conjugated anti-rabbit IgG secondary antibody (Dionova, Germany) was used to label the pY1173 primary antibody for 3 hours at room temperature, with a final 5-fold wash in Staining Buffer. Fluorescently-labelled pY1173 was measured using an Olympus FV-1000 laser scanning confocal system, mounted on an Olympus IX-81 inverted microscope (Germany). A 50 mW diode pumped solid state laser (DPSS) was used for 561 nm excitation using a UPLSAPO 60 \times /1.35 oil immersion LSM objective (Olympus). Fluorescent emission at 567 nm was detected by single plane imaging in free-run mode with a 2.0 μ s/pixel scan speed. The area mean grey values for each single cell were plotted along with population-averaged time-dependent pY1173 signals.

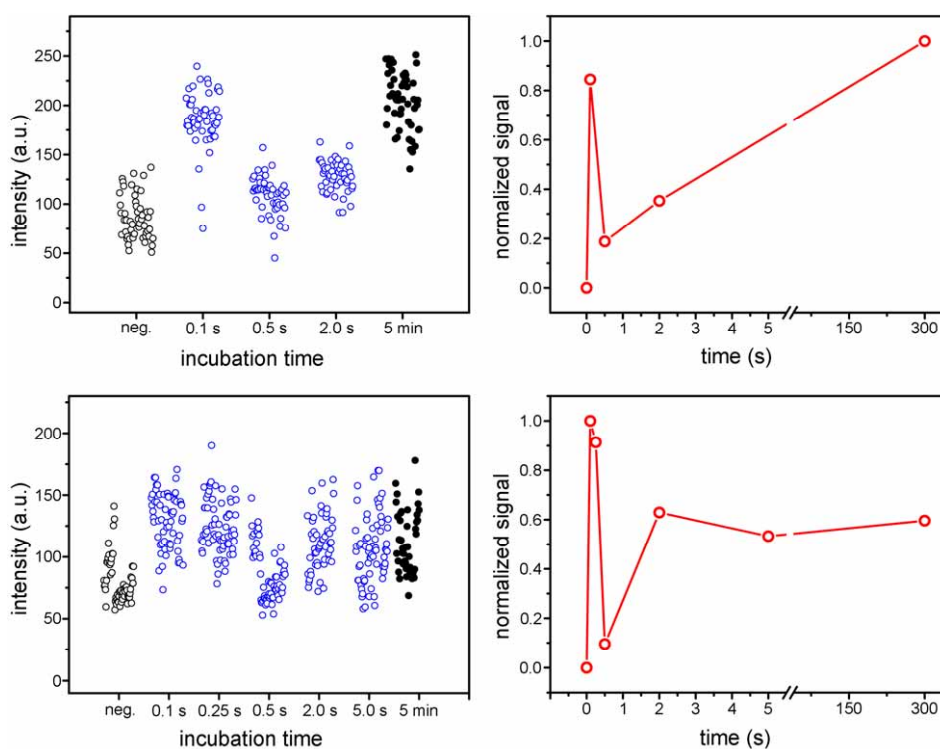
Videos

Video 1. Macroscopic mixing using a magnetically-actuated stirring element. Simulation of the methodology described by Moehren *et al.*³ Reagent homogenization requires ~1 s.

Video 2. Continuous-flow single cell pinched flow deflection into a dye doped laminar stream. The transport velocity in the STE is ~100 mm/s. The frame rate has been reduced 10-fold.

References

1. J.-P. Frimat, M. Becker, Y.-Y. Chiang, D. Janasek, J. G. Hengstler and J. West, *Lab Chip*, 2011, **11**, 231–237.
2. R. Marie, J. P. Beech, J. Vörös, J. O. Tegenfeldt, F. Höök, *Langmuir*, 2006, **22**(24), 10103–10108.
3. G. Moehren, N. Markevich, O. Demin, A. Kiyatkin, I. Goryanin, J. B. Hoek, and B. N. Kholodenko, *Biochem.*, 2002, **41**, 306–320.



ESI, Fig. 1. Replicate pY1173 transition data (for comparison see Fig. 3(B) and Fig. 4 of the main article). Single cell pY1173 signalling data obtained from preliminary microfluidic quenched-flow EGF stimulation experiments (left). Population-averaged data describing the general kinetics of pY1173 signalling (right). Absence of data for >2 s to 5 s EGF incubations prevents the diffusion-limited kinetics of EGFR dimerization being captured (top). Lengthy sample storage (3–5 days) prior to cell imaging resulted in reduced signal levels and greater signal heterogeneity within the cell populations (bottom).