Supplementary Material (ESI) for Chemical Communications

Supplementary Material (Huebner et al)

Experimental Details

Device Fabrication and Operation

Microfluidic devices were fabricated by polydimethylsiloxane (PDMS) molding from SU-8 masters. Access holes were punched into the PDMS channel ends with a 25 G syringe needle. To yield rigid microfluidic test devices, 1-mm-thick microscope slides were used as the chip-to-world interface. Holes coinciding with access holes in the PDMS microfluidic layer were drilled with a 1-mm-diameter diamond drill bit. Standard fused silica capillaries (150 µm I.D., 367 µm O.D., Composite Metal Services, Hallow, UK) were then inserted and fixed with chemically resistant epoxy to serve as fluidic reservoirs (Araldite 2014, RS Components, Corby, UK). For optical imaging the PDMS microchip was attached to a 160 µm microscope cover slip. The microchip layout comprises three inlets, a 50 µm-wide, 50 µm-deep and 7 cm-long mixing channel and a common outlet. A precision syringe pump (PHD 2000, Harvard Apparatus, Cambridge, MA, USA) was used to deliver solutions at flow rates ranging from 10 nL/min – 5000 nL/min using a 1 ml gastight syringe (SGE Europe Ltd, UK).

Detection System

The completed microfluidic device was placed on a translation stage and appropriately aligned and integrated with a custom built confocal spectrometer for fluorescence excitation and detection. Briefly the spectrometer consisted of a 488 nm CW air-cooled argon ion laser excitation source and a single avalanche photodiode detector. A dichroic mirror (505DRLP02; Omega Optical, Brattleboro, VT, US) is oriented at 45° to reflect 488 nm radiation and so define a vertical axis, normal to the surface of the optical table. An infinity corrected, high numerical aperture (NA) microscope objective (Fluar 100×/1.3 NA, oil immersion; Carl Zeiss Ltd., Welwyn Garden City, UK) brings the light to a tight focus within the microfluidic channel. Fluorescence emitted by the sample was collected by the same objective and transmitted through a dichroic mirror. An emission filter (515EFLP; Omega Optical, UK) removes any residual excitation light. A plano-convex lens (+50.2F; Newport Ltd.) focused the fluorescence onto a precision pinhole (200 µm; Melles Griot) placed immediately in front of the detector operating in single-photon counting mode (SPCM-AQR-14; EG&G Canada, Vaudreuil, Quebec, Canada). The detector dark count rate on average was approximately 100 Hz. The electronic signal from the detector is coupled to a digital to analogue converted (National Instruments), running on a Pentium PC.

Expression construct (Venus-pIVEX)

The Venus gene [Nagai, 2002 #1] was amplified from plasmid pCS2-Venus using 5' TAGATTCCATGGTGAGCAAGGGCGAGGAG 3' and 5' GCAATGGATCCTTACTTGTACAGCTCGTCCATGC 3' primers, digested with NcoI and BamHI respectively, and cloned into a modified pIVEX2.6d vector (Roche) in which the ampicillin marker had been replaced by a kanamycin marker. As this system is not under the control of any inducer the expression is accompanied by cell growth.

Cell preparation

E. coli (BL 21 DE3) cells transformed with the Venus-pIVEX vector were grown at 37 °C and 225 rpm for 16-20 h in Luria Bertani broth containing kanamycin (50 µg/mL). The cell suspension was centrifuged (3500 rpm, 10 min) and the supernatant discarded. For dilution on thip (Figure1 and 2) the resulting cell-pellet was washed (10 mL LB), and diluted with LB to an A^{600nm} of 0.55. For time-dependent measurements (Figure 3) a cell suspension in LB (A^{600nm} of 0.20) was grown (37 °C, 225 rpm) and aliquots removed (at 0, 75, 150, 255, 320 mins), its A^{600nm} determined, diluted (PBS, 1:1, v/v) and introduced into the microfluidic system.

Fluidics

Precision syringe pumps (PHD 2000, Harvard Apparatus, Cambridge, MA, USA) were used to deliver the solutions at flow rates ranging from 10 nL/min – 5000 nL/min using a 1 ml gastight syringe (SGE Europe Ltd, UK). The oil-phase consisted of light mineral oil (Sigma-Aldrich) containing 1% weight/volume Span 80® (Sigma-Aldrich). All liquids were filtered before use in microfluidics (oil phase: Minisart® 0.45 µm, Sartorius; LB and phosphate-buffered saline (PBS): Millex®GP, 0.22 µm, Millipore). Dilution on chip was achieved using one aqueous inlet stream containing the cell suspension (A^{600nm} of 0.55; LB/PBS or LB) and a second stream

containing no cells. Excellent flow stability in our experiments is confirmed by inspection of the inset in Figure 2b. The greater the variation in droplet size and droplet generation rates, the broader the full width half maximum of the Fourier transform. For the current experiments, the droplet frequency was 60 Hz with a FWHM less than 1 Hz, indicating a variation of less than 5%.

Visualization of Cells in Droplets

Figure S1 shows a photograph of a section of the microchannel containing droplets. The image was taken using a Phantom V7.2 camera (10 bit SR-CMOS 800 x 600 pixel sensor, monochrome) at 60x magnification. Individual cells localized within discrete droplets are marked with white circles.

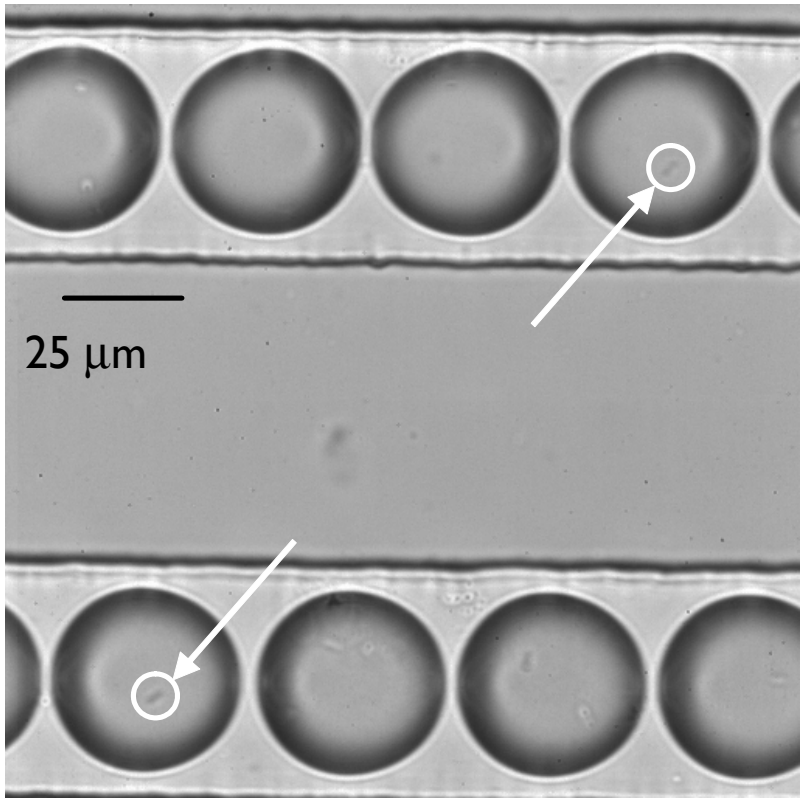

Figure S1