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# **Supporting Information**

Differential Detection Photothermal Interferometry for Fast and Sensitive Absorbance Detection in Individual Microdroplets

> Richard M. Maceiczyk, David Hess, Flora Chiu, Stavros Stavrakis and Andrew J. deMello

## S1 Droplet Time Trace



Figure S1: DC-Signal (dotted red line) and corresponding photothermal time trace for the  $44 \,\mu$ mol/L experiment. The signal magnitudes are scaled to facilitate comparison. In reality the DC signal is approximately 30 times larger than the photothermal signal. The spikes at the droplet edges visible in the photothermal signal result from interference in the measurement bandwidth when the droplet oil interface passes through the detection point.

## S2 Chip Designs and Experimental Details



Calibration and 10 kHz Experiments

Figure S2: Design for the calibration and 10 kHz experiments. The red arrow indicates the point where the absorbance measurement is performed.

At the detection point the channel is 35  $\mu$ m high and 50  $\mu$ m wide. Droplets with a volume of approximately 100 pL are formed at frequencies between 700 and 1100 Hz using a flow focusing geometry. The oil phase consists of two parts FC-40 containing 1% Raindance surfactant (RD) and one part 1,3-Bis(trifluoromethyl)-5-bromobenzene, resulting in an refractive index close to that of the aqueous phase. The calibration curves are obtained for Erythrosin B in Tris buffered, ultra-pure water (2 mmol/L, pH 9). The analyte concentration in the droplet is adjusted by dissolving Erythrosin B in the buffer (100, 20, 10, 1  $\mu$ mol/L) and diluting on-chip with pure buffer. For the experiment involving a droplet frequency above 10 kHz, a 20  $\mu$ mol/L solution of Methylorange is used to provide sufficient absorbance of the 460 nm diode laser beam.

#### **Droplet Reinjection Experiment**



Figure S3: Design for the droplet reinjection experiment. The two black triangles indicate the point where the absorbance measurement is performed.

A standard droplet generation device is used to create different populations of droplets containing 0, 20, 40, 60, 80 and 100  $\mu$ M Erythrosin B in Tris buffered, ultra-pure water (2 mmo/L, pH 9). The droplets with a volume of 150 pL are produced at approximately 3 kHz using two parts HFE 7500 with 2% RD and one part 1,3-Bis(trifluoromethyl)-5-bromobenzene. Syringe pumps are programmed to periodically produce droplets of a certain analyte concentration for one minute each for a total of one hour. Droplets are collected in a 1 mL eppendorf vial containing 100 L mineral oil to prevent evaporation. The collected droplets are withdrawn into a tube connected to a syringe filled with oil and subsequently transferred into a microfluidic device (channel height 50  $\mu$ m, width 30  $\mu$ m) via displacement pumping. The tube is arranged to direct the flow against gravity to concentrate droplets for reinjection using their buoyancy.

#### Femtoliter Droplet Experiment



**Figure S4**: Top: Design for the femtoliter droplets experiment. Illustrated in red is the mask for the detection point and in black the mask for the interface. The blue arrow indicates the point where the absorbance measurement is performed. Bottom: Three dimensional scan of the droplet generation and detection section obtained with a Keyence microscope.

For the production of fL-Droplets a two layer design is employed. The section where droplets are produced and detected has a height of 4.2  $\mu$ m and a width of 5.0  $\mu$ m. The remaining parts of the chip feature a height of 25  $\mu$ m to reduce pressure. The oil phase consisted of silicone oil (20 cst) containing 0.1% Span 80, while the aqueous phase was a mixture of Tris buffer (2 mmol/L, pH 9) 47% w/w and glycerol 53% w/w with 47  $\mu$ mol/L Erythrosin B. Droplets with a volume of approximately 150 fL are formed at a frequency of above 1 kHz. Pressure based pumps are used to accurately control the flow rates in the order of a few nanoliters per minute.

Beta-Galactosidase Colorimetric Assay



Figure S5: Design for the colorimetric assay to determine the kinetics of the reaction of beta-Galactosidase with Chlorophenol Red-beta-D-galactopyranoside. The red arrows mark the interrogation points where the absorbance measurement is performed.

Droplets containing a fixed amount of beta-Galactosidase and different concentrations of chromogenic substrate Chlorophenol Red-beta-D-Galactopyranoside are created and the content of the droplets mixed using a wiggly channel structure. The oil phase consists of FC-40 containing 1% RD and three aqueous solutions are composed of pure buffer, buffer containing 3 U/mL beta-Galactosidase and buffer containing 1 mmol/L Chlorophenol Red-beta-D-galactopyranoside (PBS buffer: pH 7.4, Mg<sup>2+</sup> and Ca<sup>2+</sup> free). Droplets with a volume of approximately 120 pL are produced at a frequency of 150 Hz and subsequently pass several equally spaced detection points corresponding to equally spaced reaction times. The initial reaction rates are determined by linearly fitting the photothermal signal of the first four points to obtain a Michaelis-Menten plot. Through fitting of the Michaelis-Menten equation the Michaelis-Menten constant is extracted.

#### Single Cell Activity Assay



Figure S6: Design for the single cell activity assay. The red dot marks the interrogation point where the absorbance measurement is performed. The dimensions of the channel at the interrogation point are  $40 \times 55 \,\mu\text{m}$  (w × h).



**Figure S7**: Left: Cell distribution in the droplets obtained from photographs. The total number of droplets analyzed is 1814. The expected value is 0.23 as obtained via fitting of a Poissonian distribution. Right: Photograph of droplets directly after formation and encapsulation of cells.



**Figure S8**: Michaelis-Menten plot of the reaction rate against substrate concentration for the reaction of beta-galactosidase and chlorophenol-red-beta-D-galactopyranoside.

The experiment was performed in a Fluoromax-4 spectrofluorometer equipped with an absorbance detector. The reagents were rapidly mixed in a 1 mL polystyrene cuvette and immediately inserted into the spectrometer. The reaction was performed at room temperature in phosphate buffered saline (pH 7.4) at an enzyme concentration of 0.03 U/mL (Sigma Aldrich, Lot #SLBF5960V) and variable amounts of substrate. Absorbance at 532 nm was detected in 0.1 s intervals up to 20 s after initiation of the reaction.

## S4 Bulk Cell Proliferation Assay



**Figure S9**: Absorbance at 450 nm against cell concentration for the WST-1 cell proliferation assay at different incubation times.

HL-60 cells were cultured in RPMI 1640 medium (Invitrogen, UK), supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen, UK), 2 mM L-glutamine (Invitrogen, UK), 50 U/mL penicillin and 50 g/mL streptomycin (Invitrogen, UK). The cell cultures were sustained in 5% CO2 humidified atmosphere at 37 °C.

A standard 96-well tissue culture plate (TPP Techno Plastic Products AG, Switzerland) was used to carry out the assay. To each well of column 3-12, 100  $\mu$ L of cell solution was added with increasing cell concentrations from row A to H (A:  $1 \times 10^4$  cells/mL; B:  $8 \times 10^4$  cells/mL; C:  $1.5 \times 10^5$  cells/mL; D:  $2.2 \times 10^5$  cells/mL; E:  $2.9 \times 10^5$  cells/mL; F:  $3.6 \times 10^5$  cells/mL; G:  $4.3 \times 10^5$  cells/mL; H: 5 × 10<sup>5</sup> cells/mL). As a control, 100 µL of pre-warmed RPMI medium (without Phenol Red; Invitrogen, UK) was added to each well of column 1-2 instead of cells. Subsequently 10 µL of Cell Proliferation Reagent WST-1 was added to each well of column 5-12 at different time points to allow an end-incubation period of 0.5, 1, 2 and 4 h for column 5-6, 7-8, 9-10 and 11-12 respectively. As a second control, 10 µL of pre-warmed RPMI medium was added to each well of column 3-4 instead of WST-1 reagent. For column 1-2, 10 µL of reagent were added to each well. Furthermore, for the control experiments, the reagent (column 1-2) or medium (column 3-4) was added to the corresponding well at different time points, to create a range of end-incubation periods: 0.5 h in the case of row G-H, 1 h in E-F, 2 h in C-D and 4 h in A-B. The plate was incubated in 5% CO2 humidified atmosphere at 37 °C accordingly. The EnSpire<sup>®</sup> Multimode Plate Reader (PerkinElmer, USA) was used to take absorbance measurements of each well at 460 nm.