Simultaneous measurement of reactions in microdroplets filled by concentration gradients

(Supplementary Information)

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1. Measurement of speed of microdroplets in the parallel channels

We loaded the microfluidic device with certain flow rates (Q) of water (W), green dye (GD), red dye (RD) and oil (see Table S1), and measured the average speed (v) of ten microdroplets in each of four channels. The variation speed of microdroplets was characterized by the coefficient of variation (CV), defined as $CV = \sigma/v$, where σ is the standard deviation.

We repeated these measurements for various flow rates of these four liquids. Table S1 summarizes the measurements.

Table S1 Speeds of microdroplets (v) and coefficients of variance (CV_1, CV_2, CV_3, CV_4) of microdroplets obtained in each of four parallel channels and for the entire population of microdroplets ($v_{1\text{-}4}$ and $CV_{1\text{-}4}$, respectively).

Q_W	Q_{GD}	Q_{RD}	Q_{OIL}	($\mu L \cdot h^{-1}$)	v_1 CV_1	v_2 CV_2	v_3 CV_3	v_4 CV_4	$v_{1\text{-}4}$ $CV_{1\text{-}4}$	($mm \cdot s^{-1}$)
0.5	0.5	0.5	25		0.29 0.018	0.31 0.017	0.31 0.021	0.32 0.020	0.31 0.023	
10	10	10	50		0.88 0.018	0.91 0.017	0.91 0.021	0.92 0.020	0.90 0.022	
50	50	75	150		2.55 0.018	2.58 0.018	2.57 0.020	2.62 0.019	2.57 0.023	
100	100	100	150		6.44 0.020	6.46 0.021	6.47 0.021	6.49 0.022	6.45 0.024	

We observe the speed did not change significantly from channel to channel. Because of the small difference between the mean speeds of microdroplets generated in the parallel channels the value of CV for the entire population of microdroplets was approximately 1–2% higher than the value of CV for the microdroplets generated in the individual channels. The value of CV for the entire population of microdroplets did not exceed 0.024, and did not significantly change with the flow rates.

Movie S1 PμD technology demonstrated using food dyes: speed of microdroplets = 2.57 $mm \cdot s^{-1}$; volume of microdroplets = 20 pL. (This experiment corresponds to the third line of Table S1.).

2. Construction of the standard curve (fluorescence *vs* product concentration) for microdroplets in the parallel channels

To generate the standard curve, we loaded the microfluidic device with buffer, product (fluorescein 15 µM or 3.75 µM in buffer), enzyme (*E. coli* alkaline phosphatase 1.5 µM in buffer) and oil and formed microdroplets. The fluorescence emission of the microdroplets was measured along different points of section S_3 and is plotted in the following graph. The combined data were fitted to a sigmoidal curve by nonlinear regression (KaleidaGraph software) and the equation was used to calculate the product concentration from the time courses shown in Figs. 6A-C.

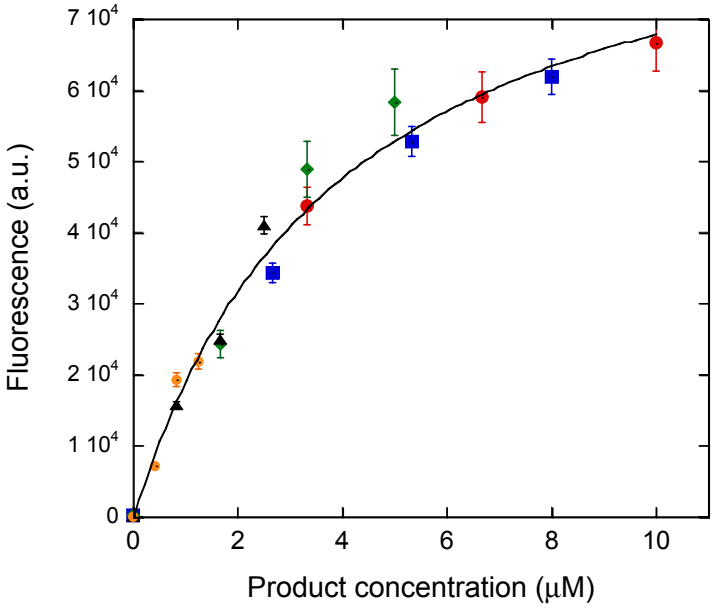

Fig. S1 Fluorescence detection in the PµD device. Each set of data corresponds to specific flow rates: ● = 10, 10, 10, 10 (for oil, buffer, fluorescein 15 µM and *E. coli* alkaline phosphatase respectively, expressed in µl·h^{-1}), ■ = 10, 8, 8, 14 and ◆ = 10, 5, 5, 20; ▲= 10, 10, 10, 10 (for oil, buffer, fluorescein 3.75 µM and *E. coli* alkaline phosphatase respectively, expressed in µl·h^{-1}).