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

Supplementary data

Measurement of chamber dimensions via UV auto fluorescence of SU-8 and by AFM

FI of the structure's UV auto-fluorescence is correlative to the height of the chamber walls.

Figure S1 shows the UV fluorescence image of an fL DSC array. A profile line based curve generated from FI values along an arbitrary horizontal line is shown in Figure S1b.

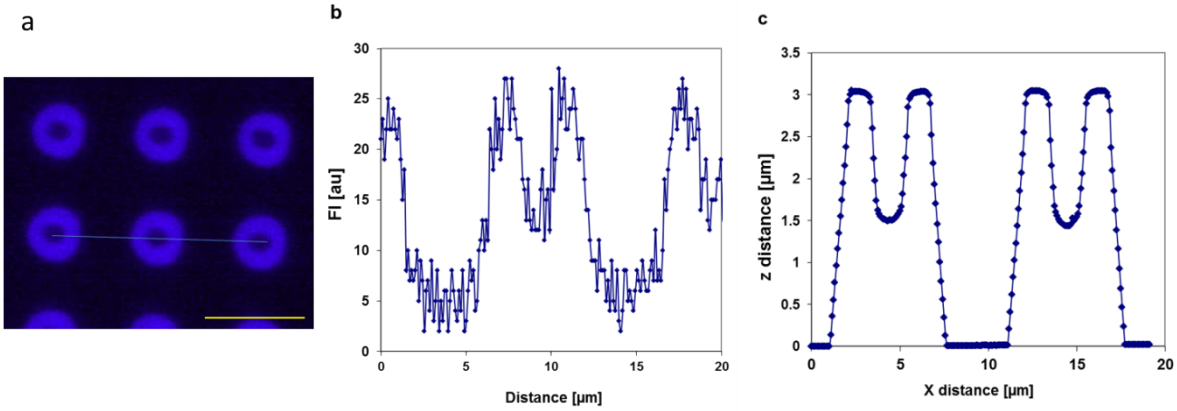

Figure S1

Measurement of chamber dimensions via UV auto-fluorescence of SU-8 and by AFM.

a UV auto-fluorescence image of an fL DSC array. Scale bar 10µm.

b Profile line of UV FI values along an arbitrary line.

c Profile line of chamber dimensions along x and z axes as measured by AFM.

The effect of volume estimation error on measurement of enzyme reaction rates within DSCs

Given that Molt-4 and UCB used in this study are spherical, as are most blood cells in suspension [Cecilia Di Ruberto, Andrew Dempster, Shahid Khan, Bill Jarra. Image and Vision Computing, 20, (2) 2002, 133-146], cell diameter could be extracted via two immediate procedures:

(a) directly measuring the radius on the largest 2D image projection (S) by crossing S with a few profile lines and counting the number of pixels along them, from which the cell volume $V_c = \frac{4\pi}{3} r^3$ is calculated.

(b) defining the cell as a Region of Interest (ROI), from which, utilizing Olympus Cell^P software, the area S_{ROI} of said ROI is calculated.

In the latter case, $r = \sqrt{\frac{\pi r^2}{\pi}} = \sqrt{\frac{S_{ROI}}{\pi}}$, from which the cell volume (V_c) could be estimated as

$$V_c = \frac{4\pi}{3} r^3 = \frac{4\pi}{3}\left(\frac{S_{ROI}}{\pi}\right)^{\frac{3}{2}} = (4/3) {S_{ROI}}^{3/2} \pi^{-1/2}$$

Both procedures yielded similar results. For instance, for a given cell, 9 direct measurements of the diameter of the round image yielded $r = 8.076 \pm 0.115\ \mu m$ and $r = 8.206 \pm 0.0938\ \mu m$ (p=0.23) when calculated from 10 assessments of S_{ROI}. Whichever procedure is used, one should note the fact that volume coefficient of variance (CV_V) is related to the radius coefficient of variance (CV_r), as

$$CV_V = dV/V_C = 3(4\pi r^2/3)dr/(4\pi r^2/3) = 3dr/r = 3CV_r$$

In other words, the CV_V of cell volumes which are calculated from the measured radii is three times higher than that of the latter, $CV_r = dr/r$. Obviously, this broadening (error) feature, which is associated with volume estimation, confines the resolution of the proposed approach, especially when inter-sample resolution is of interest, as is described in paragraph 1 below. In practice, volume estimation error influences the assessment of the individual cell enzymatic activity which is corrected for the relative volume of a cell. The correction factor (CF) is defined as the ratio $CF \equiv V_C/V_D$, where V_D is the donut volume, and considered constant. Then, $d(CF) \equiv dV_C/V_D$, and hence the CV of the correction factor is the same as that of V_C, since $d(CF)/CF = dV_C/V_C$. Next,

1. Whether performing direct, or ROI-based measurements of r, for each cell at least 9-10 repetitive measurements were performed, yielding 10 data points (defined as the sample – S), an average volume $\bar{V}_S$ (defined as the arithmetic average of the sample), a corresponding standard deviation of the sample ($SD - \sigma_S$) and $CV_S = \sigma_S/\bar{V}_s$. In these measurements, CV_r never exceeds 1.4%, and hence theoretically, CV_V never exceeds 3·CV_r=4.3%. Indeed, as said above, this result limits the resolution of the proposed approach in general, but in particular when the issue of interest is inter-sample differentiation, i.e. differentiation between samples (cells) of the same type. Hence, practically in such cases, the enzymatic activity of two cells should differ from each other by at least 4.3% in order to be resolved.
2. On the other hand, when the goal is distinguishing between populations (each made of a set of samples), then the population average (P_{av}) and the standard error of the mean σ_P (SEM – the SD of the mean) are the parameters of interest. In such case, σ_P can be evaluated from a sample by dividing the standard deviation σ_S (obtained from repetitive volume measurement of the same cell) by the square root of the sample size n_S (assuming statistical independence of the values in the sample):

$$SEM = \sigma_P = \frac{\sigma_S}{\sqrt{n_S}}$$

Hence, the coefficient of variance $CV_{p,V}$ of population cell volumes would be

$$CV_{P,V} = \frac{\sigma_P}{\bar{V}_P} \cong \frac{\sigma_S/\sqrt{n_S}}{\bar{V}_S} = \frac{CV_{S,V}}{\sqrt{n_S}}$$

Therefore, in the worst case scenario of this study,

$$CV_{P,V} = \frac{4.3\%}{\sqrt{9}} \cong 1.4\%$$

which is narrow enough for satisfactory inter-population resolution, as seen with Molt-4 and UCB populations.

Measuring purified enzyme activity in fL DSC

Figure S2 shows the reaction rates of purified esterase from porcine liver, measured at three different enzyme concentrations within fL DSC. Increasing enzyme concentration resulted in a linear rise in FDA hydrolysis rate. The rate of fluorescein production was 0.199, 0.652 and 1.734 µM/min for 0.5, 2, and 5 enzyme units respectively (R^2 = 0.998).

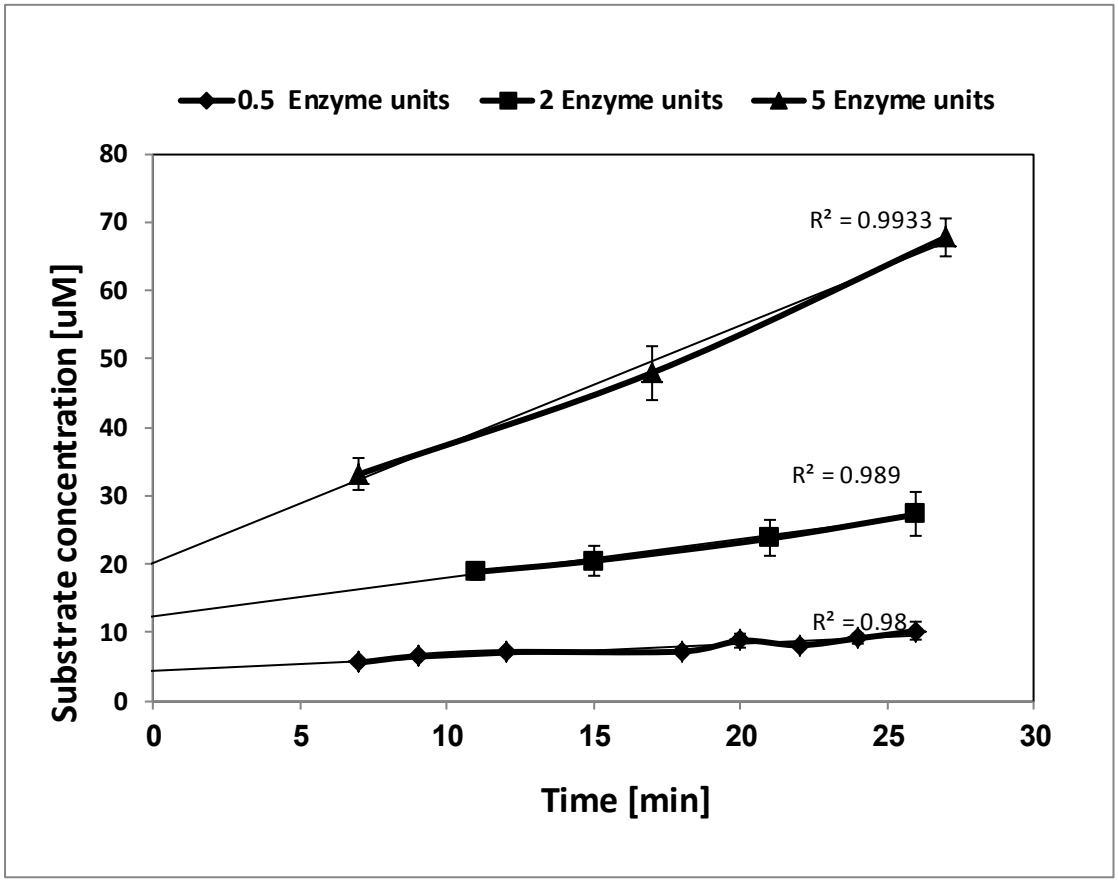

Figure S2

Measurement of enzymatic activity of purified esterase.

FI(t) of fluorescein measured during FDA hydrolysis within fL DSC using three concentrations of purified esterase. Each dot represents mean ±SD of 200-300 individual fL chambers.