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

Supplementary information

List of Supplementary Videos:

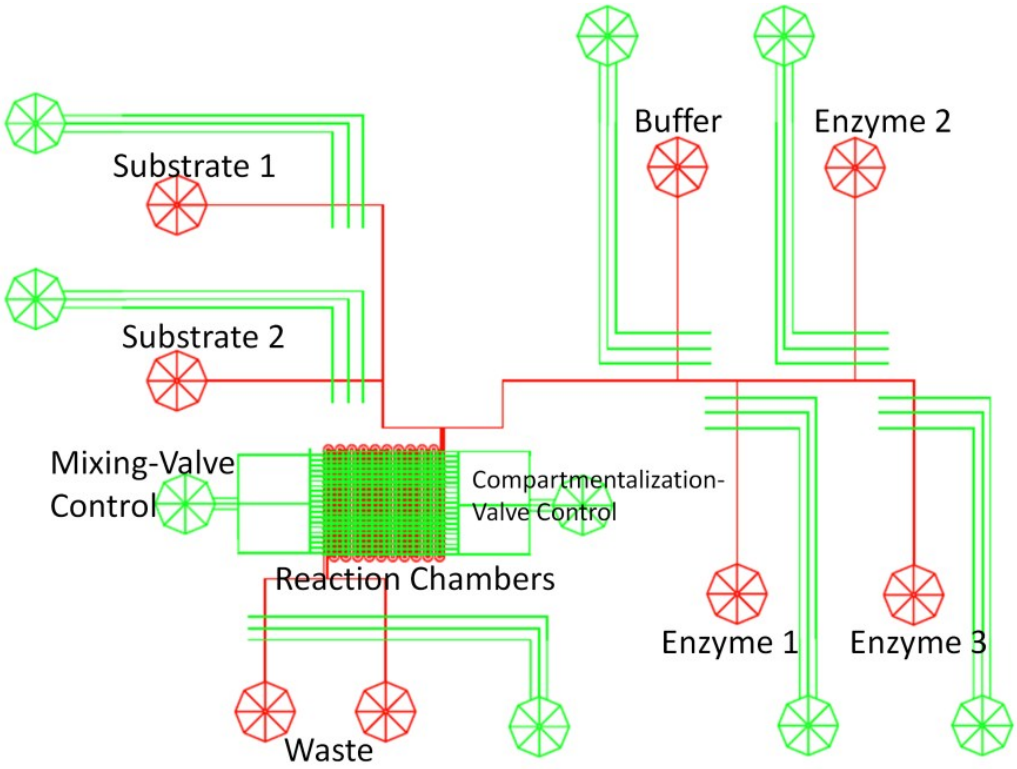
Supp Movie 1_4fM images.mov: The 100 images taken consecutively in 100 measurements of 4fM β-gal enzyme concentration is shown. Each image is taken at the end of 5 minutes reaction time.

Supp Movie 2_Channel filling.mov: Injection of two different liquids to two independent flow channels is shown.

Supp Movie 3_Valve Action.mov: Transition between the three states of the valves is shown. At first, the mixing valves (MVs) are closed (used for loading/priming the chip); then the compartmentalization valves (CVs) are closed (to trap reagents/beads before starting the reaction); finally, MVs are opened to start reaction.

Supp Movie 4_Liquid extraction.mov: Purging the contents of the channel is shown.

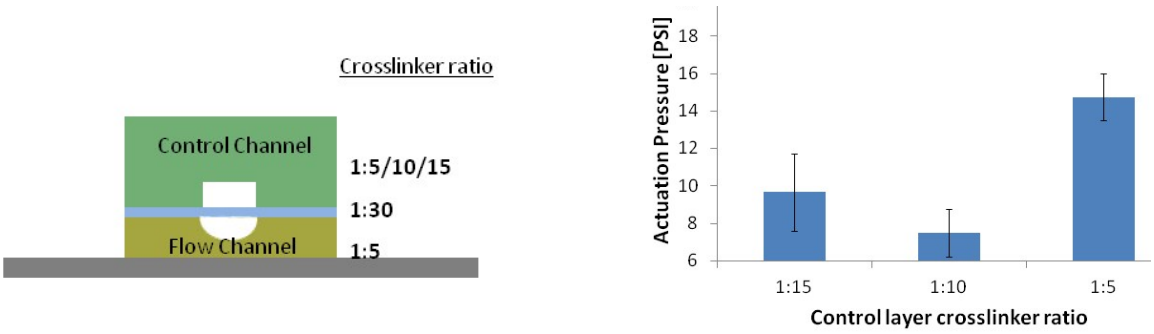
1. Chip AutoCAD design:



Supplementary Figure 1: Chip Outline. The red lines indicate the flow channels and green lines indicate the control channels. The Inlets are shown on top and outlets are shown at the bottom of the figure.

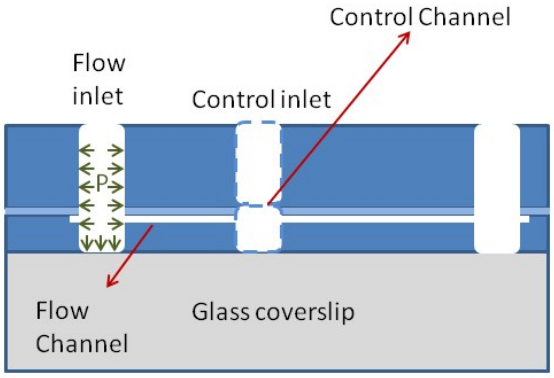
There are two substrate inlets on the left for two different substrate concentrations. There are four different enzyme inlets on the right for four different enzyme concentrations (or for buffer). There are two outlets, one for substrate and one for the enzyme. The grid in the middle is the measurement area where 400 reaction chambers reside.

2. Fabrication protocol:

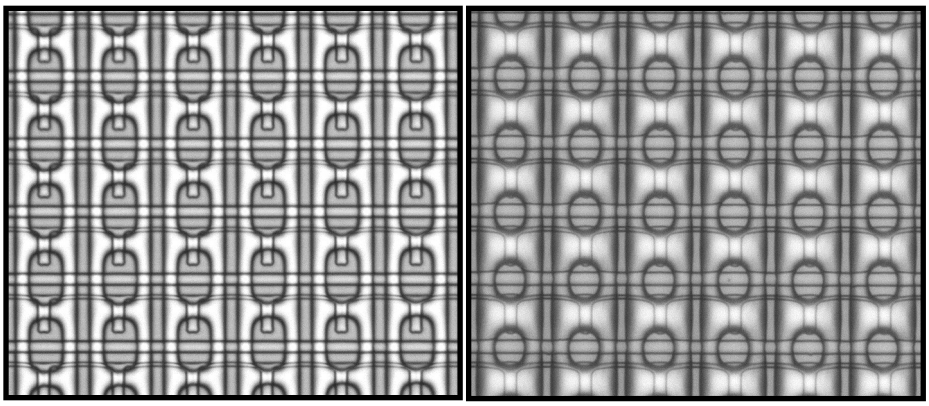


a) b)

Supplementary Figure 2 a) Three layer device architecture showing the PDMS cross-linker ratio in each layer. b) The effect of control layer cross-linker ratio on the actuation pressure. The error bars show the standard deviation of three different fabrication procedures

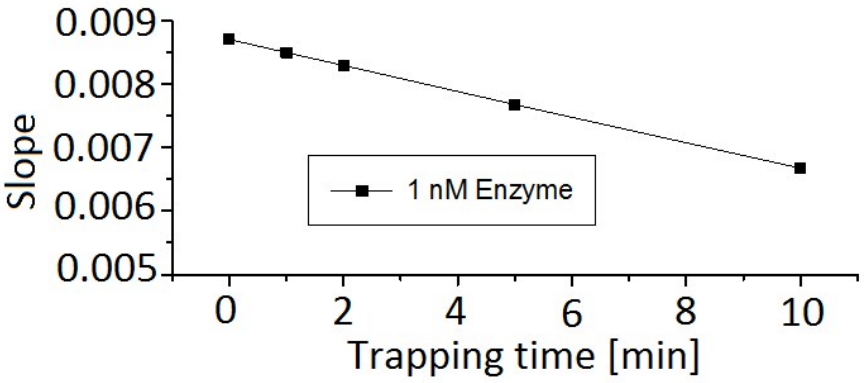


Supplementary Figure 3 The device cross section from the side view showing inlets. Punching the control and flow inlets at the end of the bonding procedure eliminates the clogging of the channels and de-lamination.

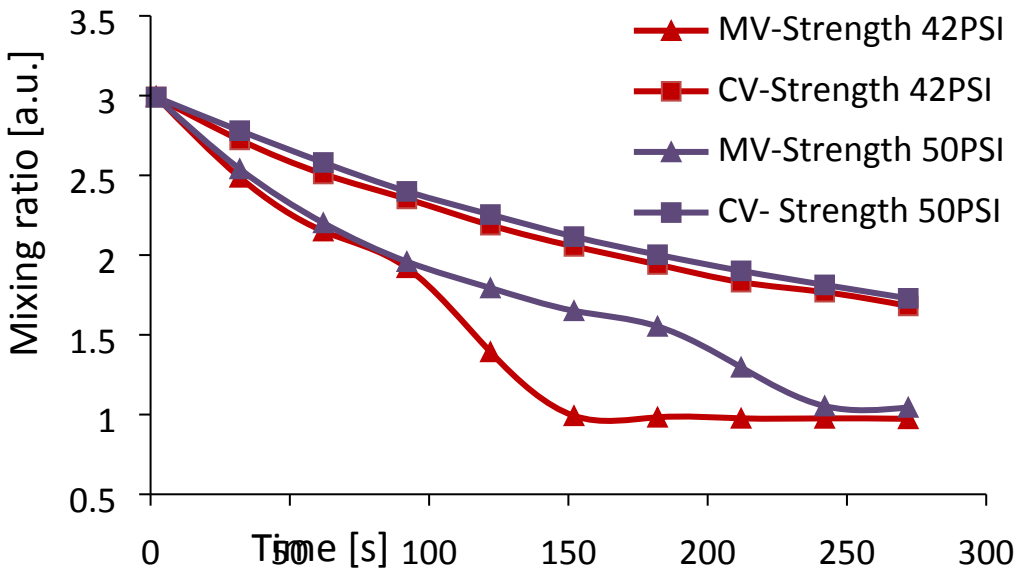


Supplementary Figure 4. The bright field image of the chip with 3 µm height (left) and 4 µm height (right). The width of the channels was 8 µm in both cases. The increase of the mixing/reaction channel height and width in the right is due to the reflow characteristics of photoresist

3. Enzyme/substrate adsorption and PDMS permeability:

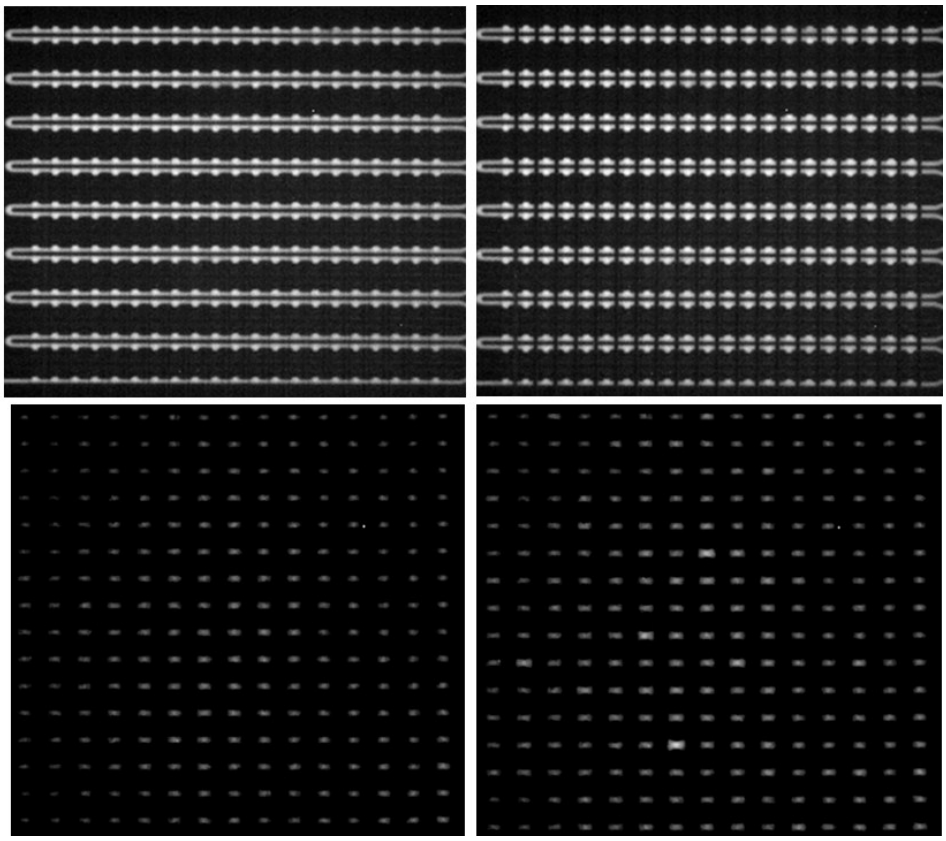


Supplementary Figure 5. Enzyme activity reduction due to trapping in reaction chambers. The y-axis is the positive fluorescence slope showing the enzyme activity. The enzyme chamber is trapped for the indicated time periods in x-axis while substrate channel is continuously flowing. At t=0 the substrate chamber is trapped and mixed with the enzyme chamber and the fluorescence signal increase (slope) in the first 30 seconds is measured. This plot shows that approximately 10% of the enzymes trapped in picoliter PDMS reaction chambers loose functionality in five minutes.

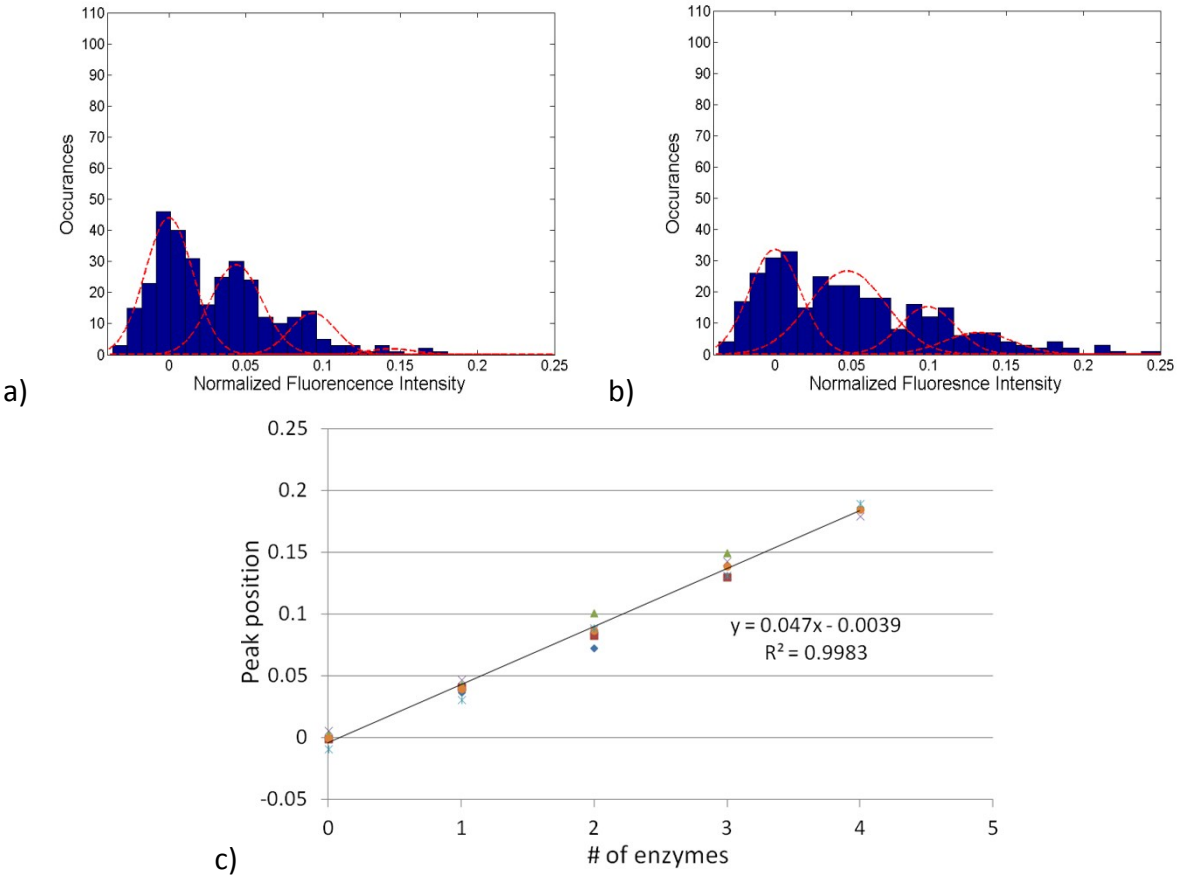


Supplementary Figure 6. The comparison of the valve behavior for mixing valves (MVs) and compartmentalization valves (CVs) for two different operation pressures (42 PSI vs 50 PSI). The buffer and substrate channels are separated first by MVs (to characterize MVs) and then by both CVs and MVs (to characterize CVs). The ratio of fluorescence intensity in buffer and substrate chambers are plotted to quantify crosstalk between chambers. The main reason for the crosstalk is the PDMS permeability. However the fact that, chambers that are separated by CV's has significantly less crosstalk indicates that valve dimensions play a role in crosstalk. The kink at 100th (200) second for 42 (50) PSI valve operation is due to the spontaneous valve opening and instant mixing of enzyme and substrate channels. This is due to the large height of the MVs but does not deteriorate chip performance as the MVs are always open during reactions.

4. Chip Operation and Enzyme detection

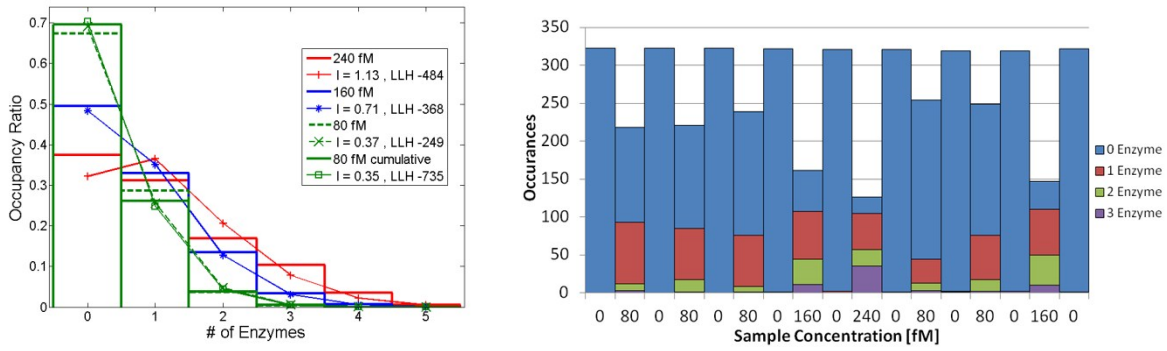


Supplementary Figure 7. Fluorescence microscope images of the detection area while there is substrate and sample loaded in the flow channels; (top, left) Mixing valves (MVs) are closed, compartmentalization valves (CVs) are open; sample and substrate flowing in parallel. (top, right) MVs and CVs both are closed; sample and substrate trapped (bottom, left) CVs are closed, MVs are open; buffer and substrate mixed (image taken at the end of 5 minutes) (bottom, right) CVs are closed, MVs are open; 4 fM enzyme (~6 enzymes in average) and substrate mixed (image taken at the end of 5 minutes).



a) b) c)

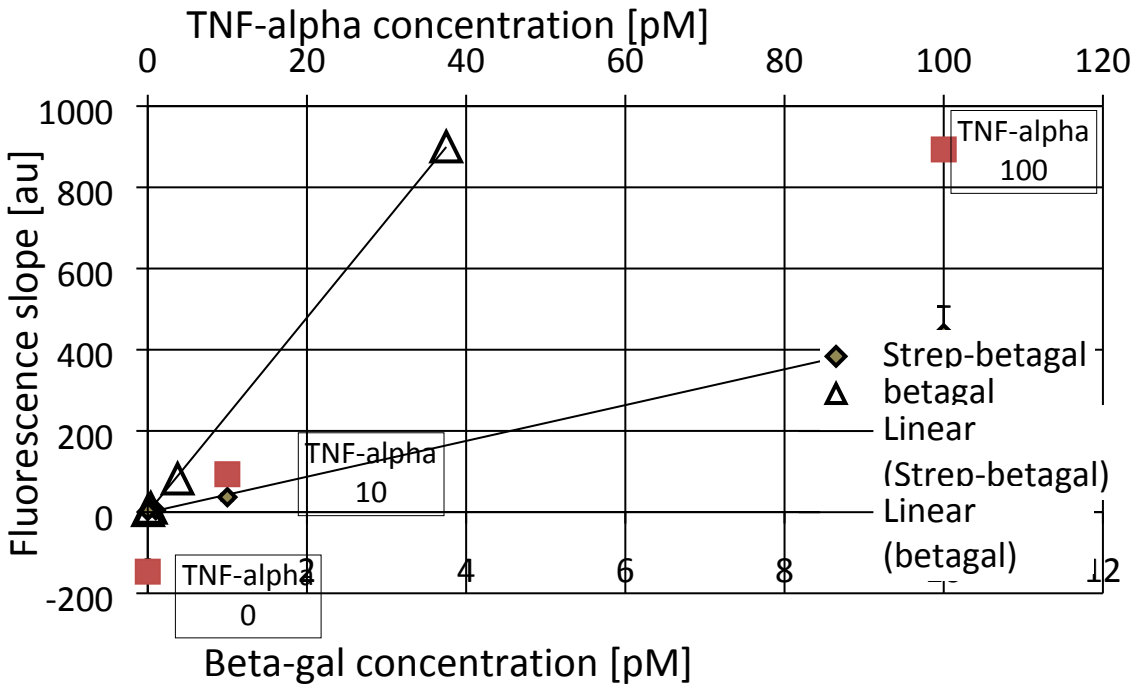
Supplementary Figure 8. Histograms of the normalized fluorescence intensity for a) 160 fM and b) 240 fM β-gal. A Gaussian curve is fit to each peak. c) Position of the peaks obtained from 5 experiments.



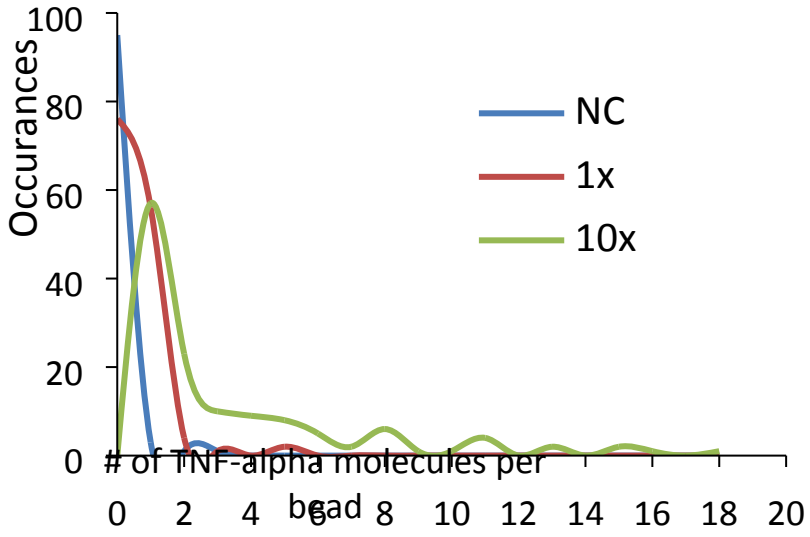
a) b)

Supplementary Figure 9 a) The ratio of the area under each peak to total number of chambers (bar graph) and their corresponding Poisson fit (line curves) for 80 fM (green), 160 fM (blue) and 240 fM (red) β-gal concentrations. b) Demonstration of reusability for improving the dynamic range; successive measurements of buffer and various enzyme concentrations.

5. TNF-α detection using magnetic beads



Supplementary Figure 10. Strategene measurement results for purified β-gal, streptavidin-β-gal conjugate and magnetic beads that are prepared according to the protocol provided in online methods. The streptavidin-β-gal conjugate has almost haft the activity of pure β-gal which is due to the difference in the purity.



Supplementary Figure 11. Measured number of enzymes (corresponds to TNF-α molecules) per bead for each sample. Average number of enzymes for NC - 0.08; 1X - 0.55; 10X - 3.44 per bead.