Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION FOR:

On-Chip Gradient Generation in 256 Microfluidic Cell Cultures: Simulation and Experimental Validation.

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Table 1: Parameters used for all simulation experiments.

	Study (Flowrate -mL/hr)	Micro Channel Length (μm)	Buffer		Dye/H2O2	
			Density (kg/m^3)	Dynamic Viscosity (Pa.S)	Concentration (mol/m^3)	Diffusion Coefficient /(m^2/s)
1	Flow Rate 0.01	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
2	Flow Rate 0.02	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
3	Flow Rate 0.04 (Base Case)	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
4	Flow Rate 0.06	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
5	Flow Rate 0.08	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
6	Flow Rate 0.10	300	997	9.00E^{-04}	4.20E-^{09}	4.25E^{-10}
7	H_2O_2 Flow	300	997	9.00E^{-04}	3.26E-^{05}	1.50E^{-09}

The concentration of $c(x, y, t) = c_{in}$ at the inlet S_1, and $c(x, y, t) = 0$ at the inlet S_2 for all simulations. In all experiments the initial concentration of the dye $c_{in} = 4.2$ x 10^{-9} mol/m^3. We set the normal component of the flux of concentration to be equal to zero ($D\nabla c \cdot \vec{n} = 0$) on S_4. Here $\vec{n}$ is outward normal to the boundary S_4. The outlet boundary of S_3 is free and we model this boundary condition by the boundary equation of $D\nabla c \cdot \vec{n} = c$ on S_3. The Dynamic viscosity $\mu = 9$ x 10^{-4} Pa.s is set to the viscosity of ideal water, and the diffusion coefficient of fluorescein $D = 4.25$ x 10^{-10} m ^{2}/s.

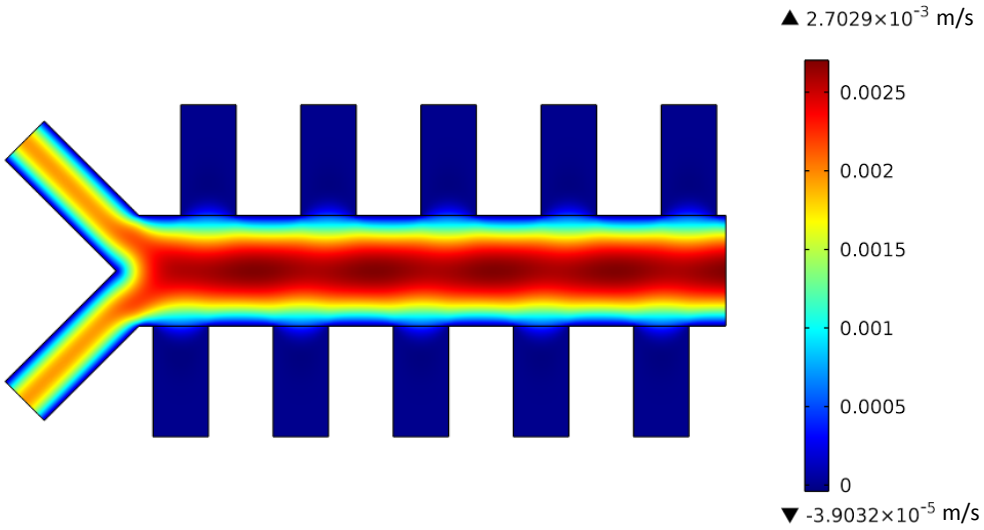


Figure S1: Velocity profile of the selected region (***R***) of the domain of flow (Flow rate on the inlets 0.04 mL/hr). Velocity vector in side chambers become zero and the highest velocity shows at the middle of the main channel. There is no fluid flow into side chambers other than circulation.

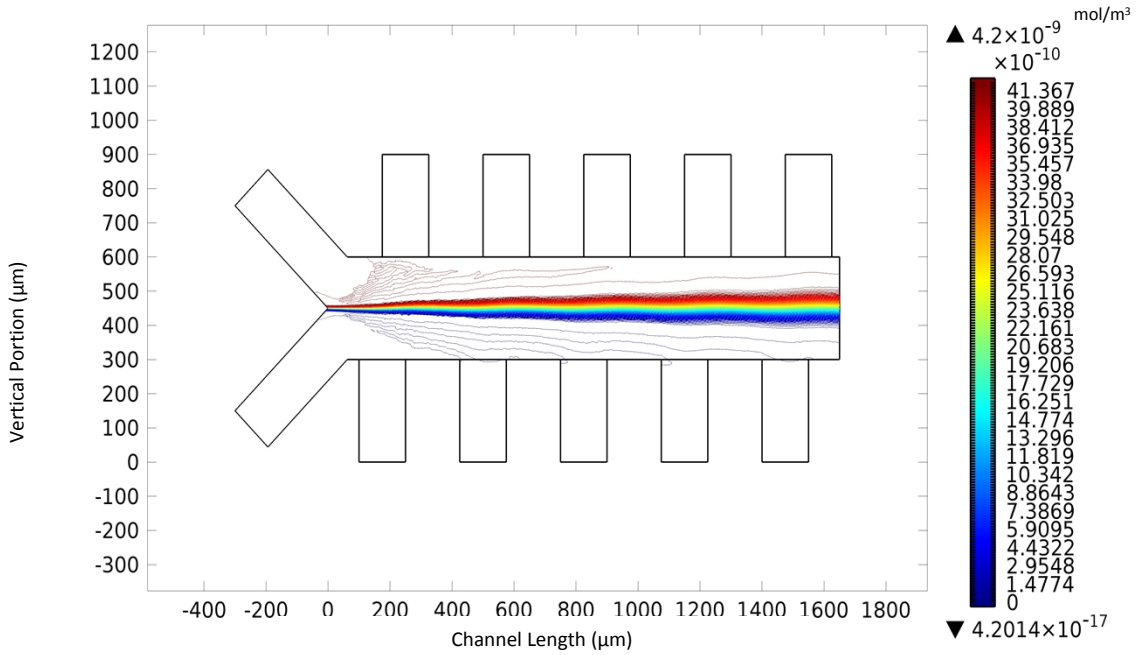


Figure S2: 2D distribution of the concentration inside domain of the flow (isolines for concentration function in the domain). Dye concentration of S_1 inlet side decreased while dye

concentration increased for S_2 inlet side. Initial coencentration of dye at inlet S_1 is 4.2 x10^{-10} mol/m^3. Concentration of each culture chamber varies from 4.2 x10^{-10} mol/m^3 to 0.

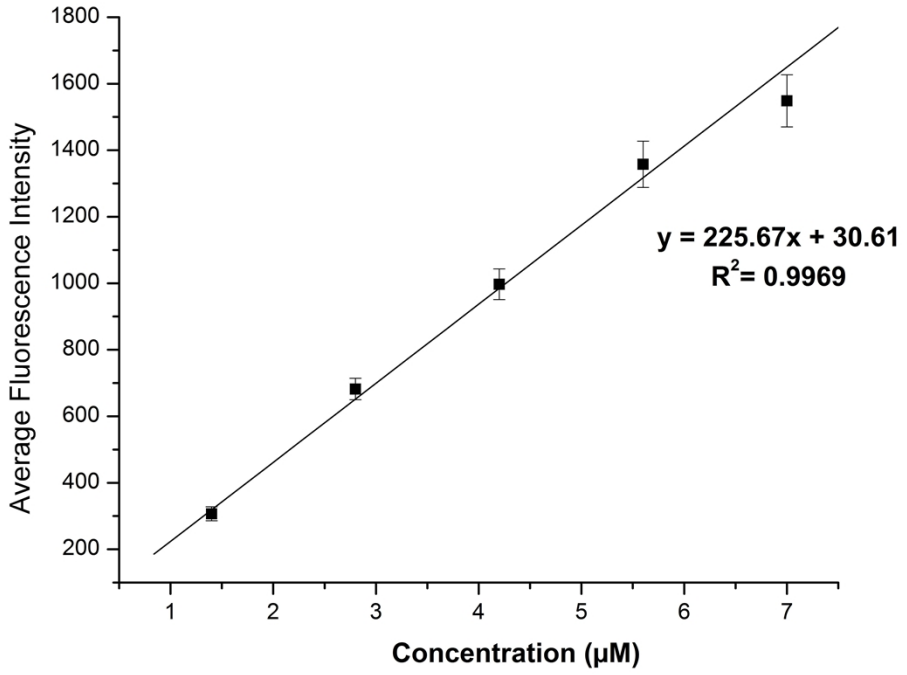


Figure S3: Calibration curve for the microfluidic diffusion diluter using fluorescence intensities of fluorescein dye. A series of fluorescein dye concentration was made and each concentration flowed through the microfluidic device one after the other under non- gradient condition and fluorescence intensities were measured. Average intensity for each concentration was used to develop the calibration method. Then an unknown sample was flowed through the device and measured fluorescence intensities.

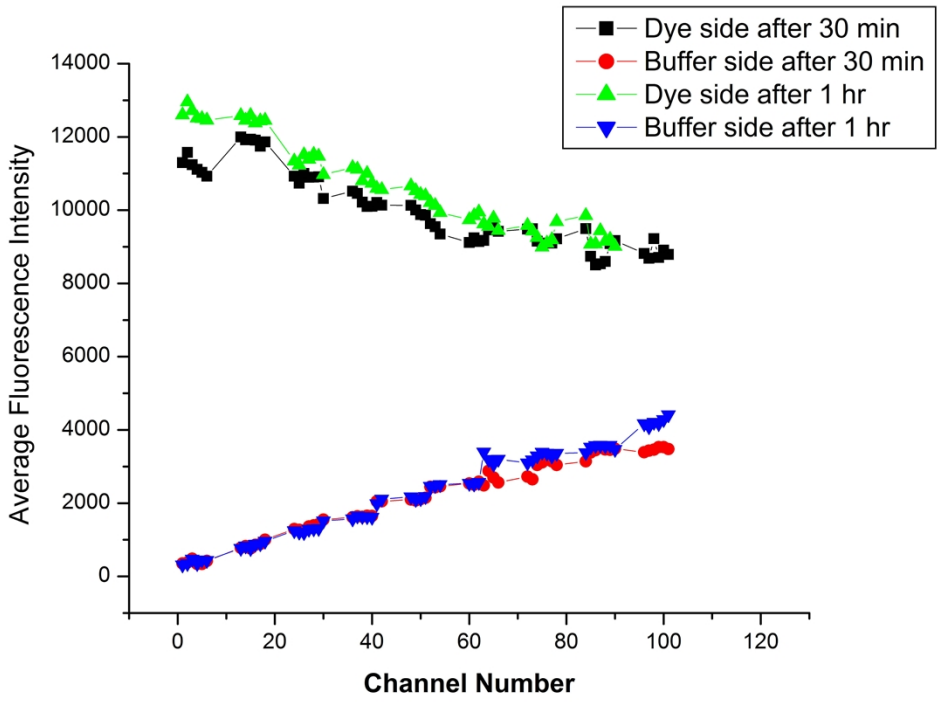
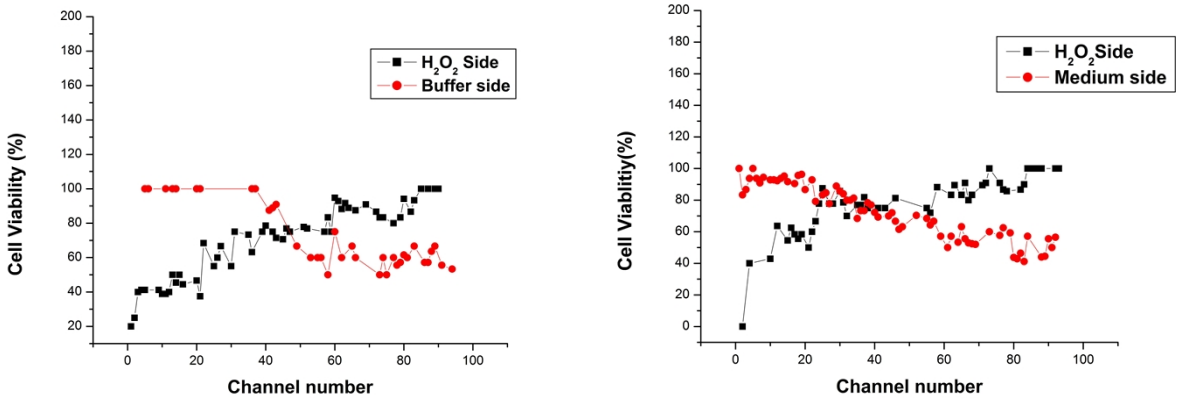
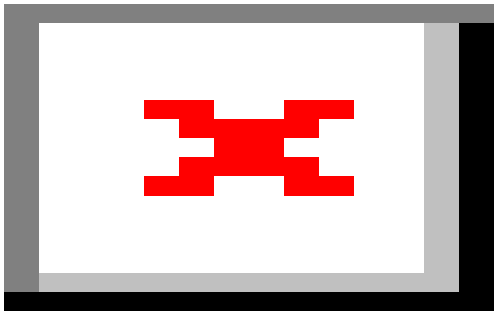


Figure S4: Relationship of intensity of fluorescein dye with channel numbers at the same 0.04 mL/hr rate. Here length of the microchannels is 0.75 mm. The gradient was established in less time (30 min) than in longer side channels which are 1.0 mm long (45 min) [22].

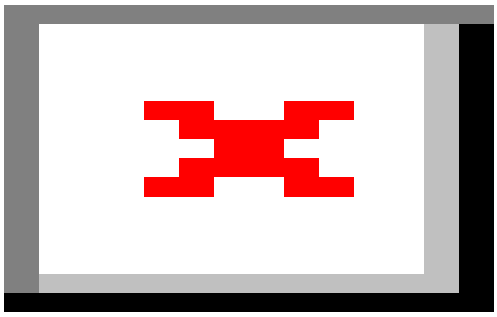


(A) (B)

Figure S5. Cell viability assay of Ramos cells stained with Calcein AM (3μM) due to concentration gradient of H_2O_2 {Initial concentration is 1%(w/v)}. Increasing viability showed in the inlet side flowed H_2O_2 (Black). Decreasing of viability showed in inlet side that flowed only RPMI culture medium (Red). Data representing (A)Trial 1 on left, (B)Trial 2 on right.



(A)



(B)

Figure S6: Simulation results of gradient formation in microfluidic devices with 0.3 mm (A) and 1mm (B) long microchannels. The legend indicates the time taken by each channel to come to steady state. 0.3 mm long channels required 500 s and 1.0 mm long channels required 1000 s. Longer the channel take more time to become steady state or equilibrate concentration gradient. Legends represent gradient run at different times starting from 25 seconds.

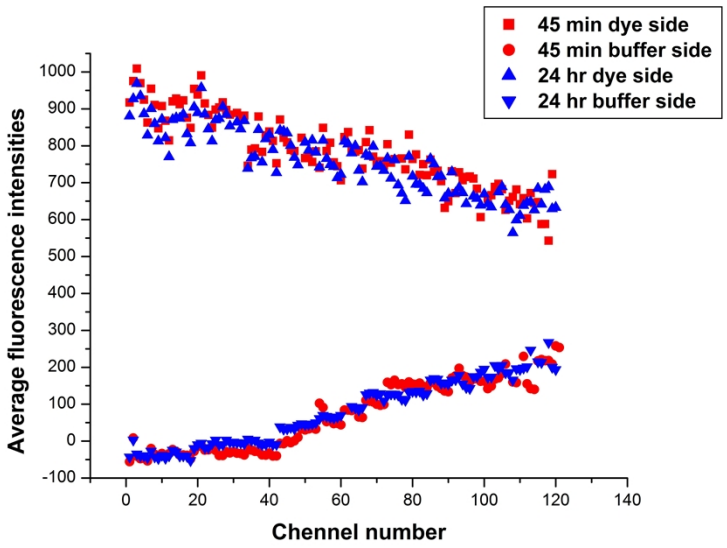


Figure S7: Relationship between fluorescence intensity of fluorescein dye versus each microchannnel in the device. Initial concentration of the fluorescein dye was 4.2 μM and flow rate was 0.04 ml/hr. Fluorescence images were taken at 45 min and after 24 hrs of continuous flow of reagents. Data represent fluorescence intensity of dye side at 45 min (red square), buffer side after 45 min (red circles), dye side after 24 hrs (blue upward triangles) and buffer side after 24 hrs (blue downward triangles). Gradient profiles at 45 min and 24hrs overlap each other and stable gradient formed for 24 hrs.

Videos:

Cell Loading Upper: Step 1 of the cell loading process, in which the channels on the upper part of the field of view are loaded.

Cell Loading Lower: Step 2 of the cell loading process, in which the channels on the lower part of the field of view are loaded.