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

Supplementary Information

A sharp-edge-based acoustofluidic chemical signal generator

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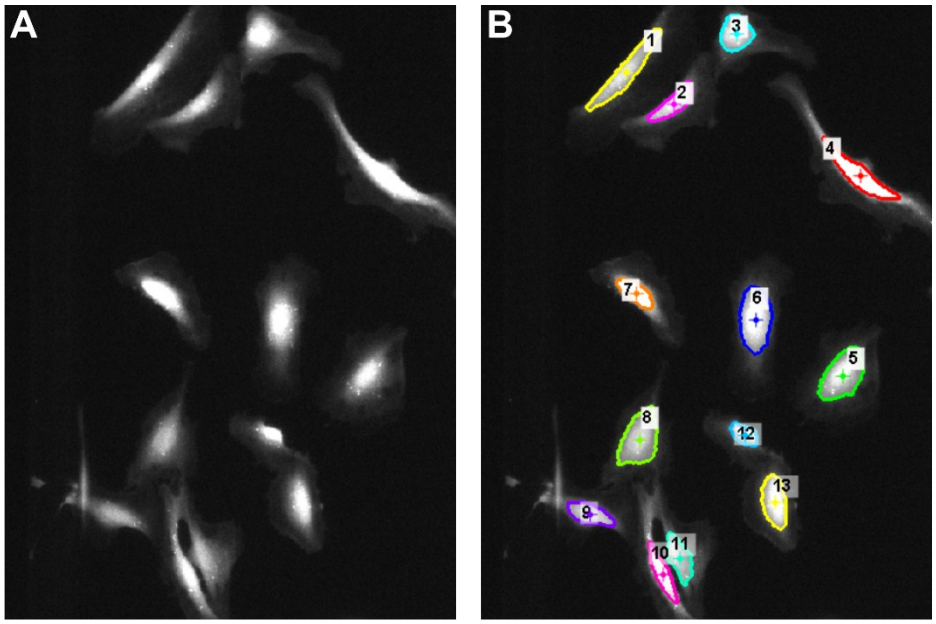

Figure S1. Images showing how the cellular fluorescence were characterized in this work. (**A**) Cells without labelling of region of interest. (**B**) Cells with labelling of region of interest which covers the cytoplasm area (at least 70% of entire cell area).

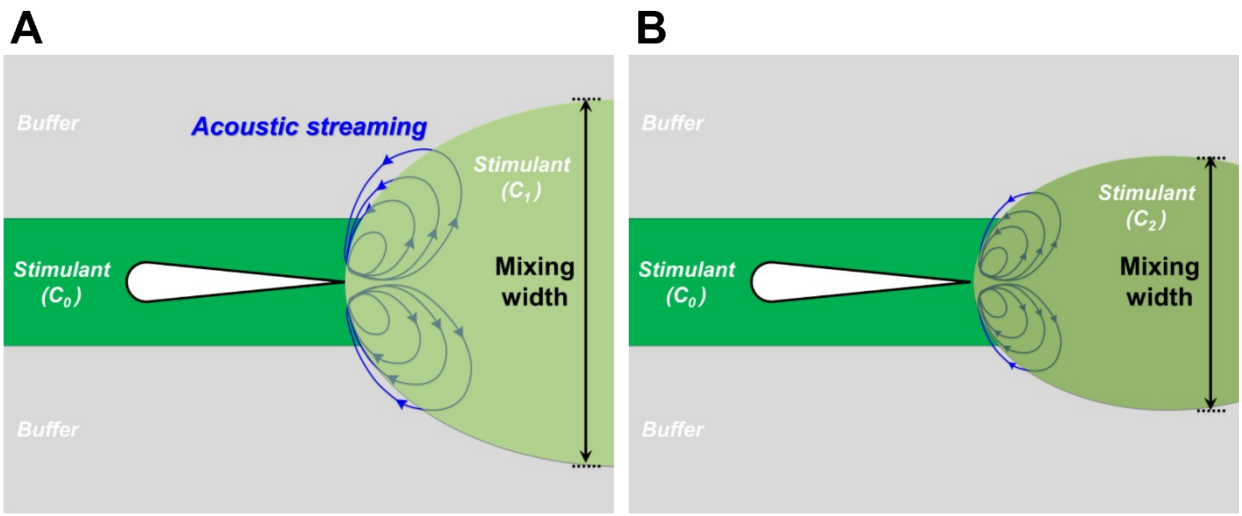

Figure S2. Concept of generating spatially controllable chemical signal. If we arrange the sharp-edge structure in the middle of the channel as opposed to the sidewall, we can generate different mixing schemes along with the modulation in driving signals of the transducer. For example, (**A**) when higher driving voltage is applied (stronger acoustic streaming), a fixed amount of stimulant (C_0) will be mixing with more buffer, yielding a heavily-diluted stimulant (C_1); (**B**) when lower driving voltage is applied, the fixed amount of stimulant will be mixed with less buffer, yielding a less-diluted stimulant (C_2).

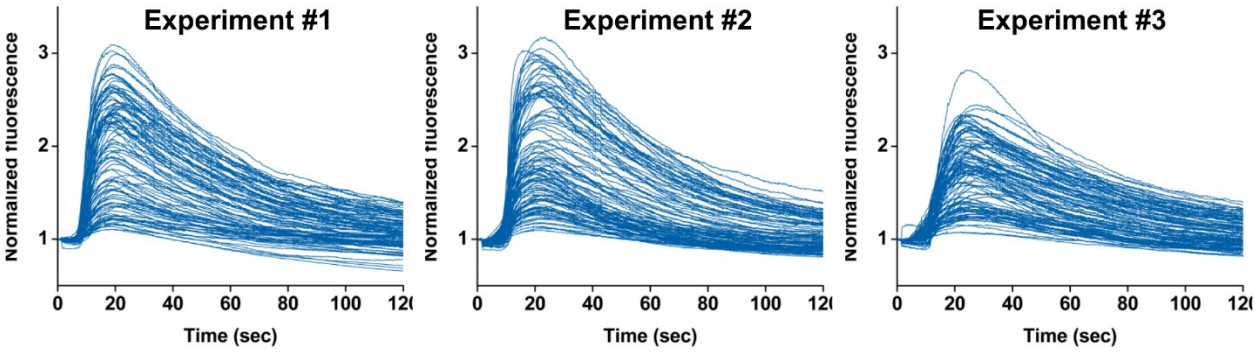

Figure S3. Plots showing the cell-to-cell variation in terms of Ca^{2+} release response under a 10 sec, single-pulse ionomycin stimulation, from three independent experiments. In each experiment, more than 100 HMVEC-d cells were examined. The cell-to-cell variation was mainly due to the heterogeneity between cells; nevertheless, the heterogeneity in this case only led to the difference in the amount of Ca^{2+} released by each individual cell. The results demonstrate that with our device, we could investigate the cell-to-cell variation in terms of cellular response, by exposing cells simultaneously to the same chemical signald.

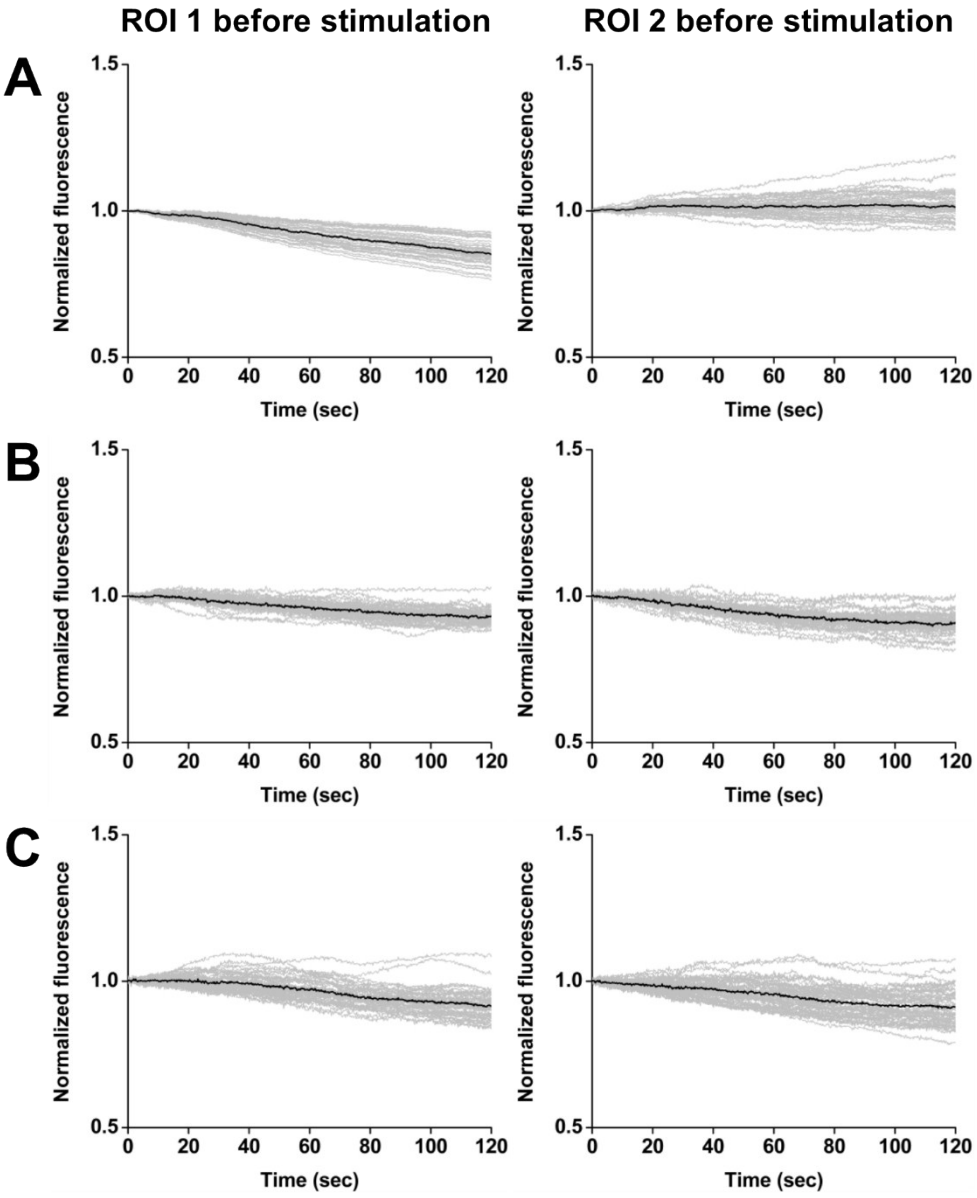

Figure S4. Individual (gray curves) and average (black curve) fluorescence intensity curves of 50 representative cells at ROI 1 and ROI 2 for (**A**) HMVEC-d cells, (**B**) HeLa cells, and (**C**) U-251 cells before the medium containing ionomycin was infused into the channel, *i.e.*, in the absence of ionomycin. No Ca^{2+} release was observed, regardless of cell type. Each plot is the representative results from at least three independent experiment where more than 200 cells in total were examined.

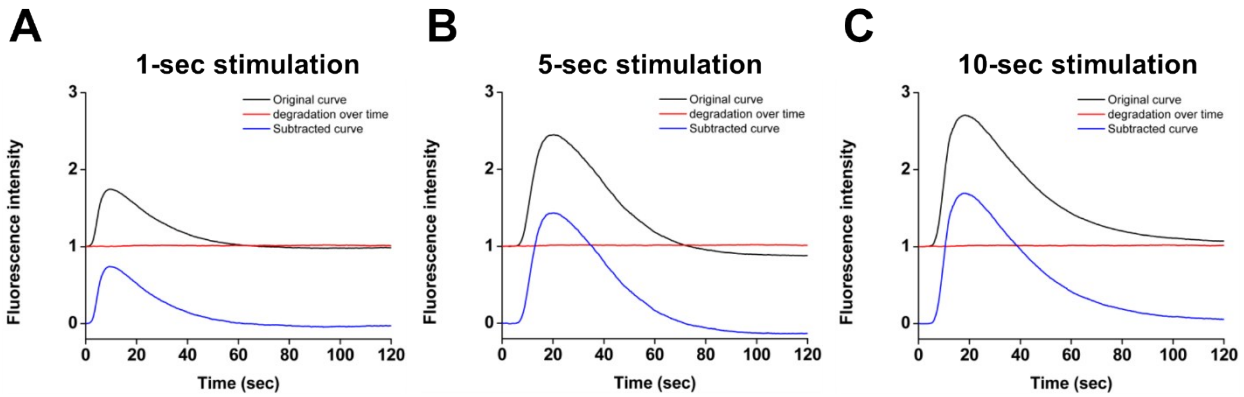

Figure S5. Plots showing the original, average fluorescence intensity curve, degradation intensity curve, and subtracted intensity curve of HMVEC-d cells under (**A**) 1-sec, (**B**) 5-sec, and (**C**) 10-sec ionomycin stimulation. Subtracting the degradation curve from the original curves, we still obtain fluorescence response curves that are similar to corresponding, original curves.

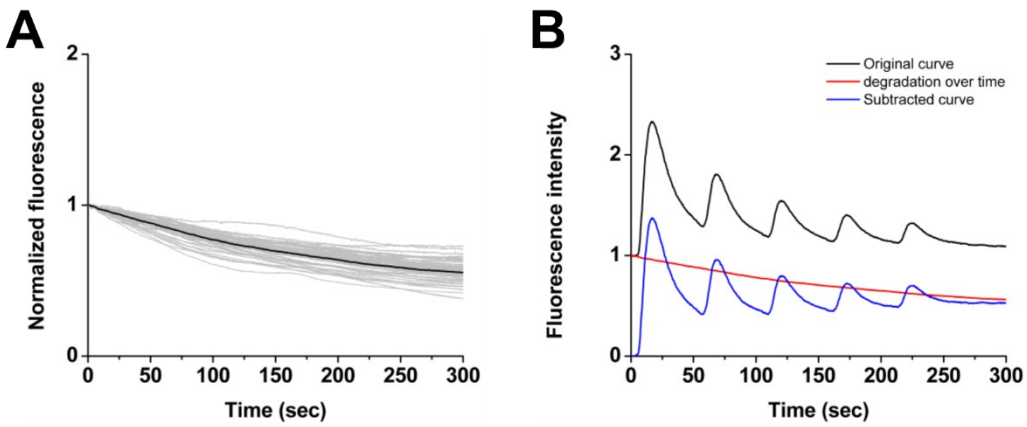

Figure S6. (**A**) Representative plot showing the Ca^{2+} response of cells initially seeded in ROI 1 under a periodic ionomycin stimulation. The result indicates that no Ca^{2+} release was observed for cells seeded in ROI 1. This is mainly due to that for the cells seeded in ROI 1, where ionomycin was always present, they constantly depleted their internal calcium stores and eventually, the calcium stores were exhausted. This is also the reason that we only presented cell responses that were collected from ROI 2. (**B**) Plot showing the original, average fluorescence intensity curve, degradation intensity curve, and subtracted intensity curve of HMVEC-d cells under 5-sec repetitive ionomycin stimulation. Subtracting the degradation curve from the original curve, we still obtaine a fluorescence response curve that is similar to corresponding, original curve.

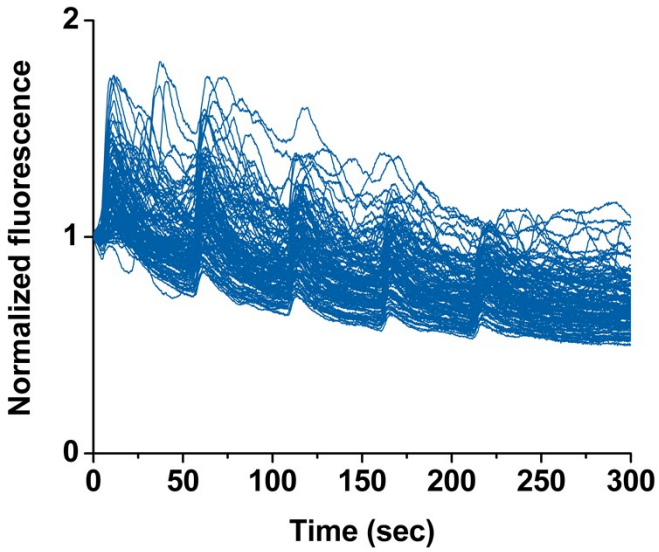

Figure S7. Plot showing the Ca^{2+} response of HMVEC-d cells challenged by a periodic ionomycin stimulation (period = 50 sec) with rapid, single-pulse stimulation profiles (100 ms). The cells exhibited multiple Ca^{2+} transients between each Ca^{2+} peak. The result, once again, suggests that when stimulated by rapid ionomycin stimulation (~100 ms), the cells underwent a process where they constantly released and accepted Ca^{2+}, respectively, from the internal calcium store (inside the cells) and surrounding environment (outside the cells).

Video Captions:

Video S1. The Ca^{2+} release response of HMVEC-d cells in response to a 5 sec, single-pulse ionomycin stimulation.

Video S2. The Ca^{2+} release response of HMVEC-d cells in response to a 100 ms, single-pulse ionomycin stimulation.

Video S3. The Ca^{2+} release response of HMVEC-d cells in response to a periodic ionomycin stimulation composed of five 5-sec, single-pulse ionomycin stimulations with a period of 50 sec.

Video S4. The Ca^{2+} release response of HMVEC-d cells in response to a periodic ionomycin stimulation composed of five 100-ms, single-pulse ionomycin stimulations with a period of 50 sec.