Figure S1. The stimulus profile visualized using FITC-BSA shows similar pattern to that of fluorescein, although FITC-BSA pattern are more dispersed compared to that of fluorescein. Profiles were generated by alternatively delivering FITC-BSA solution and PBS at 50 mHz driven by (A) 1psi, (B) 2psi, (C) 3psi and (D) 4psi. Heatmaps of spatial (Y axis) and temporal (X axis) show fluorescent intensity (color bar) in single observation chamber.
Table S1. Summary of effective dispersion analysis for fluorescein and FITC-BSA

| Driving Pressure (PSI) | Mean Flow Velocity U (m/s) | Effective Dispersion Coefficient Deff (m²/s) | Rise Time t (sec) for Row # |
|------------------------|---------------------------|---------------------------------------------|-----------------------------|
|                        |                           | 1   | 5   | 10  | 15  | 20  |
| FITC-BSA dispersion     |                           |     |     |     |     |     |
| 1                      | 5.14E-04                  | 5.67E-07 | 1.7 | 7.8 | 19.4 | 24.5 | 28.6 |
| 2                      | 1.29E-03                  | 1.73E-06 | 0.9 | 3.1 | 7.0  | 9.4  | 12.3 |
| 3                      | 1.98E-03                  | 2.82E-06 | 0.7 | 2.0 | 4.4  | 6.1  | 8.2  |
| 4                      | 2.78E-03                  | 4.36E-06 | 0.6 | 1.4 | 3.2  | 4.4  | 6.0  |
| 5                      | 3.48E-03                  | 5.35E-06 | 0.5 | 1.2 | 2.6  | 3.6  | 4.8  |

| Fluorescein dispersion  |                           |     |     |     |     |     |
| 1                      | 5.33E-04                  | 1.47E-07 | 1.1 | 6.5 | 13.3 | 15.2 | 14.2 |
| 2                      | 1.34E-03                  | 1.11E-06 | 0.6 | 2.7 | 6.0  | 7.9  | 9.4  |
| 3                      | 2.20E-03                  | 2.32E-06 | 0.5 | 1.6 | 3.8  | 5.2  | 6.4  |
| 4                      | 2.92E-03                  | 3.61E-06 | 0.4 | 1.3 | 3.0  | 4.1  | 5.1  |
| 5                      | 3.82E-03                  | 5.32E-06 | 0.4 | 1.0 | 2.2  | 3.2  | 4.1  |

*Figure S2.* The results for effective dispersion analysis are consistent across experiment repeats.
Figure S3. The choice of base pulse duration affects stimulation profile. The spatial-temporal heat map of fluorescent intensity within an observation chamber and its corresponding fluorescent intensity profile: (A) FITC-BSA solution with base pulse of 100 ms, (B) fluorescein solution with base pulse of 200 ms, and (C) FITC-BSA solution with base pulse of 500 ms.