SUPPLEMENTAL INFORMATION

Fabrication Process

As shown in Fig. S1, we first fabricated the required molds by patterning SU-8 photoresist (SU-8 10, Microchem) microstructures on two silicon wafers for the control-channel layer (thickness: 10 µm) and the micro-well layer (thickness: 50 µm). We patterned AZ photoresist (AZ 50XT, AZ electronics) on another silicon wafer for the flow channel structures (thickness: 20 µm). To facilitate release of device structural layers from the molds, we performed silanization of the molds using Trichloro (1H, 1H, 2H, 2H-perfluoro-octyl) silane (Sigma-Aldrich).

Afterwards, we prepared polydimethylsiloxaine (PDMS) pre-polymer by mixing the PDMS monomer with the curing agent at a ratio of 10:1. We degassed the pre-polymer using a vacuum chamber to remove air bubbled trapped in PDMS during mixing. The PDMS pre-polymer were then poured/spin-coated onto all the three microfabricated silicon-photoresist molds, and baked at 80 °C in an oven for the polymer cross-linking. The PDMS layers were then processed in sequence, from the upmost to the lowest layer. For each the layer, we chopped the PDMS along the device boundaries, peeled the substrates off, punched holes at the gas/liquid exits, and aligned onto the next layer of the device structures under a dissecting microscope. It should be noted that the covalent bonding between the control-channel layer and the flow-channel layer was achieved by further baking the partially cured PDMS surface together, where the bindings between the flow-channel layer and the micro-well substrate and between the micro-well substrate and the glass substrate were based on the oxygen-plasma treatment (Energy 10 kJ; Harrick plasma cleaner PDC-002).
**Step 1** Partially cure PDMS on control layer mold

**Step 2** Spincoat and partially cure PDMS on flow layer mold

**Step 3** Peel off, punch holes for the control layer PDMS; and align on the flow layer PDMS

**Step 4** Cure PDMS on micro-well mold

**Step 5** Oxygen plasma bond micro-well PDMS on glass

**Step 6** Peel off, punch holes for the control-flow layer PDMS substrate; and oxygen plasma bond it on the micro-well PDMS/glass substrate

**Figure S1** Fabrication of the microfluidic mixers.
Flow Characteristics of Device Model II

**Figure S2**  (a) Pressure inputs from inlets of the control channels for micromixer design model II. (b) Simulated pressure differences across the membranes. Profiles of (c) membrane deflection, (d) flow velocities and (e) shear stress over the chamber base of design model II, at time 50 ms, 150 ms and 250 ms in a stabilized mixing cycle.

Mixing of Dye Implemented by Device Model I

**Figure S3**  Comparison between simulation and experimental results for device model I.
Calculation of Equivalent Capacitance for the Membrane Stiffness

The membrane stiffness was estimated using the plate equations. Considering the aspect ratio of the membrane is reasonably small \((H_M/W_M \leq 0.133)\), we approximated each membrane as a thin plate with negligible thermal edge loads under a uniform normal pressure of \(V_1\), \(V_2\) or \(V_3\) for the membrane 1, 2 or 3, respectively. As discussed, the membrane deflection (<38.6 µm) is significantly smaller than \(L_M\) and \(W_M\) for both device models I and II, and therefore we neglected the in-plane displacement for a reasonable approximation. The membrane deflection \(w(x, y)\) in the Cartesian coordinates has the following relation:

\[
\frac{EH_M^3}{12(1-v^2)} \left( \frac{\partial^4 w(x, y)}{\partial x^4} + \frac{\partial^4 w(x, y)}{\partial y^4} \right) = V_i, \text{ for membrane } i \ (1, 2 \text{ and } 3) \tag{S1}
\]

where \(E (= 750 \text{ kPa})\) is Young’s modulus of PDMS and \(v (= 0.5)\) is the Poisson’s ratio of PDMS. The boundary conditions are:

\[
\begin{align*}
\frac{\partial^3 w}{\partial x^3} &= 0 \text{ at } x = 0, x = W_M, y = 0 \text{ and } y = L_M \\
\frac{\partial^3 w}{\partial y^3} &= 0 \text{ at } x = 0 \text{ and } x = W_M \\
\frac{\partial^3 w}{\partial y^3} &= 0 \text{ at } y = 0 \text{ and } y = L_M 
\end{align*} \tag{S2}
\]

Solving Eq. S1 using the above boundary conditions, the membrane deflection can be calculated as

\[
w(x, y) = \sum_{m=1,3} \sum_{n=1,3} \frac{192(1-v^2) \sin(m\pi x / W_M) \sin(n\pi y / L_M)}{mn^2 \pi^2 EH_M^3 [(m\pi / L_M)^2 + (n\pi / W_M)^2]^3} V_i \tag{S3}
\]

Integrating the above equation over the membrane area to obtain the displaced volume \(Q_i\) for membrane \(i\), we can estimated the membrane stiffness \(C\) as

\[
Q_i = C \frac{dV_i}{dt} \tag{S4}
\]

\[
C = \sum_{m=1,3} \sum_{n=1,3} \frac{768L_M W_M (1-v^2)}{m^2 n^2 \pi^4 E_M H_M^3 [(m\pi / L_M)^2 + (n\pi / W_M)^2]^3} \tag{S5}
\]
Mixing of Fluorescent Beads without Switching of Directions of the Driven Flows

**Figure S4** Mixing results of fluorescent beads implemented by the device model II. Scale bar: 200 µm. Dotted lines enclose the regions which have inefficient mixing.

Population of *S. Mutans* in a Microchamber with Mixing of the Culture Media

**Figure S5** Phase-contrast microscopic images of the *S. mutans* population growing in a microchamber at different time points (24 hr, 32 hr, 40 hr and 48 hr) with the mixing operations applied regularly over time (scale bar: 200 µm).