Supporting Information

Polymeric microfluidic continuous flow mixer combined with hyperspectral FT-IR imaging for studying rapid biomolecular events

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1. Fabrication of the MCFM platform

1a. Procedure

(I) Creation of master pattern: Negative images of the microchannels, inlets, and outlets were printed on a transparency film using a 5080 dpi printer. The patterns were transferred to an approximately 15 µm thick layer of SU-8 25 photoresist (confirmed using a Dektak 3030 profilometer) spun on a 3-inch silicon wafer at 3000 rpm via standard UV photolithography. Then a silane monolayer was passivated onto the patterned silicon surface via vapor deposition under vacuum.

(II) Spinning of PDMS fluid layer: A ~30 µm thick layer (confirmed using a Dektak 3030 profilometer) of 15:1 PDMS (weight ratio of polymer to cross-linker) was spun on the silicon master at 3000 rpm. The PDMS layer was cured on a hot plate for approximately 10 minutes at ~ 85 °C.

(III) Bonding of top COC layer to PDMS fluid layer: A 4 mil COC sheet was bonded to the PDMS fluid layer via APTMS-GPTMS bonding. The exposed PDMS surface on the silicon device and the COC sheet were exposed to oxygen plasma for about 1 minute (using Harrick Plasma, Ithaca, NY) and were immersed in 1% v/v APTMS and 1% v/v GPTMS solutions in water, respectively at 85 °C on a hot plate for approximately 20 minutes to passivate the surfaces. Then, the two substrates were removed from the solutions, cleaned with DI water and N_2, and brought into conformal contact to bond them. To reinforce the bond, the compound layer was heated for 5 minutes at 80 °C on a hot plate.

(IV) Bonding of inlet and outlet tubing to COC-PDMS layer**:** Inlet and outlet tubing were bonded to the COC-PDMS layer (on the silicon wafer) via extra PDMS. Approximately 5 mm thick PDMS blocks were prepared and the blocks were placed right before the inlets merging area and outlet hole. Then, tubing was placed on top of inlet and outlet and PDMS was poured around the tubing to hole them stably. Since the PDMS blocks were placed at the front and end of observing channel, excessive PDMS did not flow over the observing area. The tubing and COC-PDMS layer were cured in an oven for approximately 5 hours at ~ 65 °C.

(V) Fabrication of the inlets and outlets holes: The PDMS surface of the composite layer was covered with low tack scotch tape for protection. We drilled holes into the composite layer from the holes of tubing until we reached the PDMS interconnect layer. To complete the fabrication of the holes, we punched through the tubing using a 20-gauge needle (B-D Precision Slide). Then, the low tack scotch tape was removed.

(VI) Bonding of the bottom COC substrate to PDMS**:** A 4 mil COC sheet was bonded to the exposed surface of the fluid layer of PDMS-COC-PDMS composite layer via APTMS-GPTMS bonding. The exposed PDMS fluid layer surface and the COC sheet were exposed to oxygen plasma for about 1 minute and were immersed in 1% v/v APTMS and 1% v/v GPTMS solutions, respectively at 85 °C on a hot plate for approximately 20 minutes to passivate the surfaces. Then, the COC sheet and the PDMS-COC-PDMS layer were removed from the solutions, cleaned with DI water and N_2, and brought into conformal contact to bond them. To reinforce the bond, the compound layer was heated in a 65 °C oven overnight (>8h) to complete the fabrication of the device.

1b. Fabricated chip

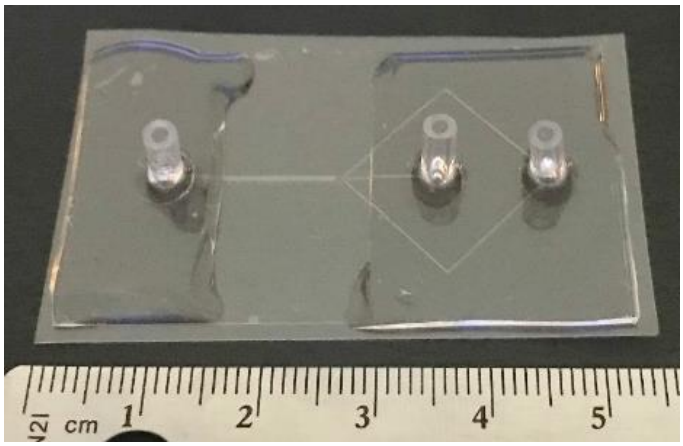

Fig. S1 Actual size and shape of MCFM after completely fabricated.

1c. Micro-CT images of the chip

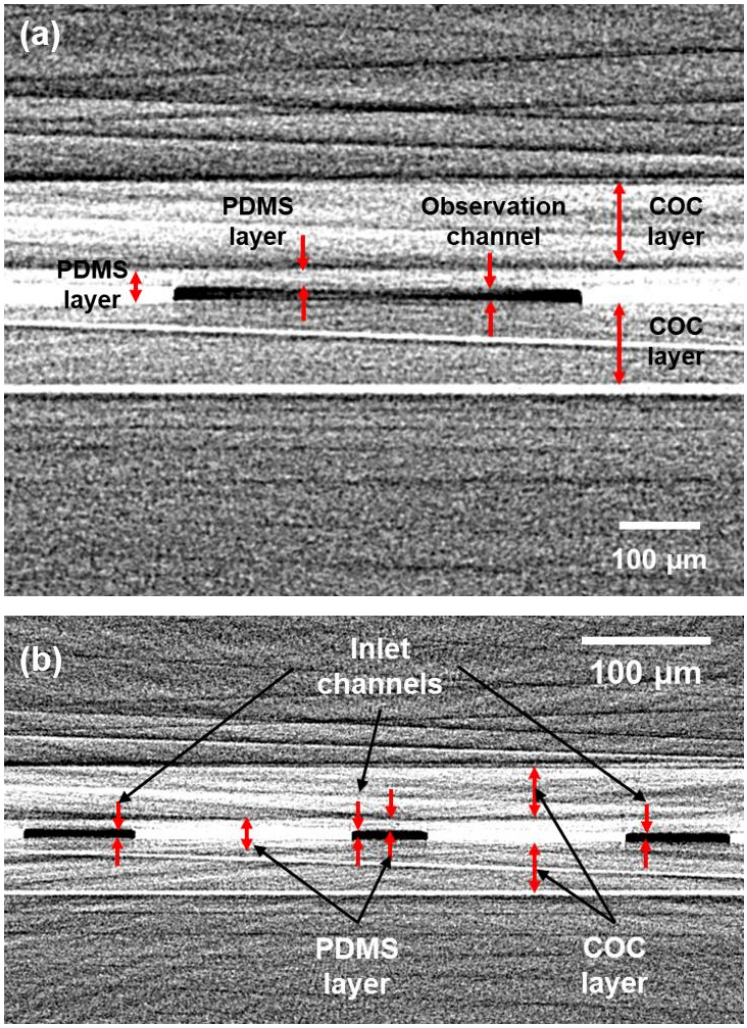

Fig. S2 *Micro-CT images of channels in MCFM.* (a) Cross-sectional view of observation channel. (b) Cross-sectional view of channels for three inlets.

2. Parameters used to acquire hyperspectral FT-IR image

Data was collected on Agilent Cary 620 FT-IR microscope with following parameters. Background spectrum was collected with empty channel (two COC layers and thin PDMS layer) on desired location before flowing solutions.

- Resolution: 4 cm^{-1}
- Number of scans: 64
- Imaging mode: Transmission
- FPA size: 128 x 128 pixel (704 x 704 μm^2)
- Objective: 15x / 0.62 N.A.
- Total time required to complete scanning: 5 minutes

2a. Full Integration of MCFM and FT-IR microscope

MCFM platform was placed on stage of microscope and connected with two syringe pumps that individually deliver solutions to center and side channels.

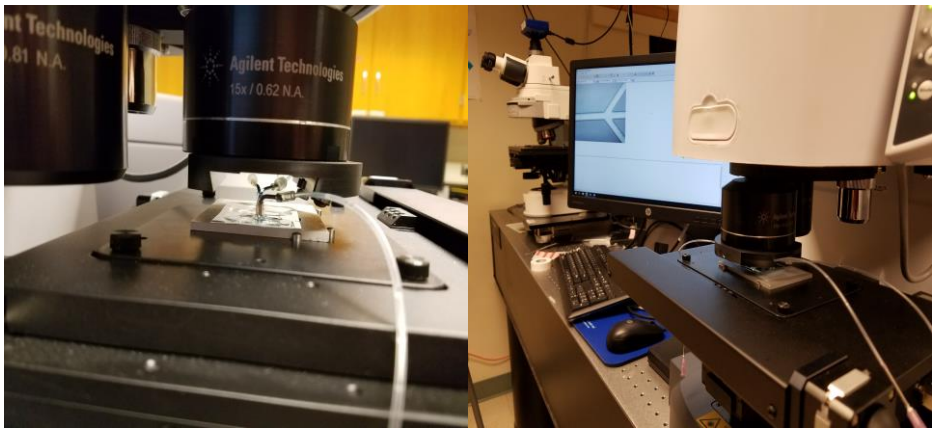

Fig. S3 Microfluidic device connected to syringe tube using L-shape connector is placed on the stage of FT-IR microscope.

3. Finite Element Analysis (FEA) simulation of H_2O and D_2O mixing

To simulate the hydrodynamic focusing and optimize the time resolution for the mixing behavior, Finite element analysis simulation (COMSOL Multiphysics Version 4.3b) was used. A 2D finite element model, steady-state, incompressible Navier-Stokes and convection-diffusion equations were applied to 'reacting flow, diluted species' study and were solved to simulate the process.

Table S1. Parameters used to simulate H_2O/D_2O mixing experiment

Parameter	Setting
Diffusion coefficient	4.5×10^{-9} m^2/s, isotropic
Central inlet concentration	55.5 M
Side inlets concentration	0 M
Material	Water, liquid
Mesh size	Extremely fine
Central inlet velocity	0.022×0.4 m/s
Side inlets velocity	0.022×2.0 m/s

3a. Velocity profiles from different angles used for broadening channel

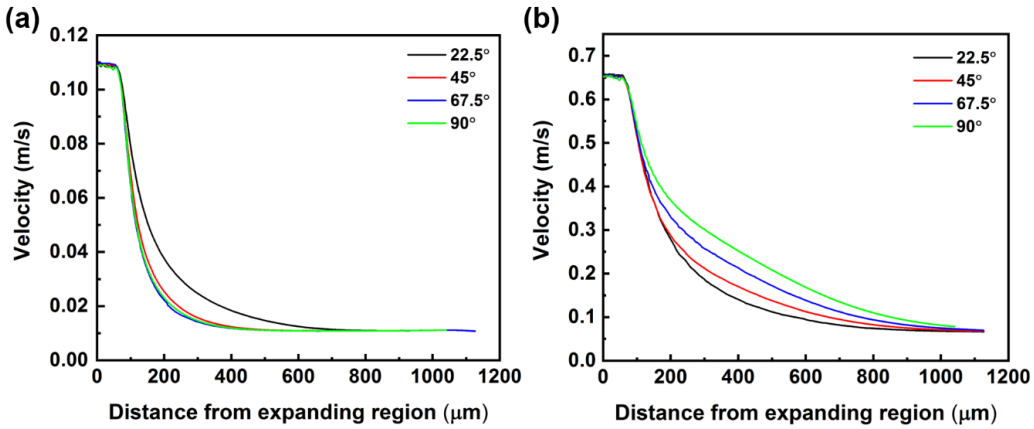

Fig. S4 *Flow speed decrement in expansion region depending on the expanding angle with different flow rate to achieve both (a) millisecond and (b) microsecond mixing times.* When flow rate is adjusted to achieve complete mixing time at millisecond, decrease in flow speed was similar for expanding angle above 45º, but it took longer when the angle was less than 45 º. On the other hand, when trying to achieve complete mixing time at sub-millisecond, decrease in flow speed took longer distance as the expanding angle increases. From these observations, 45 º was selected as an angle that can provide longest residential time in observing area to provide both millisecond and sub-millisecond mixing process.

4. Theoretically calculated mixing time

Following mass conservation,

$$w_f = \frac{Q_{center}}{V_f \times h} \quad (1)$$

$$v_o = \frac{Q_{center} + Q_{side} + Q_{side}}{w_o \times h} \quad (2)$$

$$\text{With (1)and (2),} \quad \frac{w_f}{w_o} = \frac{v_o}{v_f} \times \frac{Q_{center}}{Q_{center} + 2Q_{side}} \rightarrow w_f = \frac{v_o}{v_f} \times \frac{w_o \times Q_{center}}{Q_{center} + 2Q_{side}} \quad (3)$$

From diffusion over distance equation,

$$x^2 = q_i D t \rightarrow t = \frac{x^2}{q_i D} \quad (4)$$

Combining (3) and (4),

$$\tau_{mix} \sim \frac{w_f^2}{4D} \approx \frac{1}{4D} \frac{v_o^2}{v_f^2} \frac{w_o^2}{(1 + 2FRR)^2}$$

Q_{center}: central inlet flow rate
Q_{side}: side inlet flow rate
h: channel height
w_f: width of focused stream
w_o: width of mixing channel
v_f: average velocity of focused stream in the mixing channel
v_o: average velocity at the end of the mixing channel
q_i: constant depending on dimensionality. 2,4,6 (i=1,2,3)
τ_{mix}: mixing time
D: diffusion coefficient
FRR: flow rate ratio between side and center inlets (Q_{side}/Q_{center})

3b. The change in time-duration for observation area in accordance with the flow rates

To achieve 90% of side stream concentration in the focused stream at the end of the narrow mixing channel, flor rate ratio between side and center inlets should be 5 (Q_{side}:Q_{center} = 5:1). To calculate the mixing time, diffusion coefficient of OH^- ($5.273x10^{-9}$ m^2/s), 5 for FRR, and V_f and V_o from FEA simulation are used. With these parameters, the theoretically fastest mixing time is about 460 µs. Thus, about 4.8 ms for residence time in observation area meets two criteria that need to be considered when 2.2 µL/min for central inlet is used. Diffusion coefficient for OH^- is chosen because the change in pH for inducing biomolecular reactions is used to validate the MCFM.

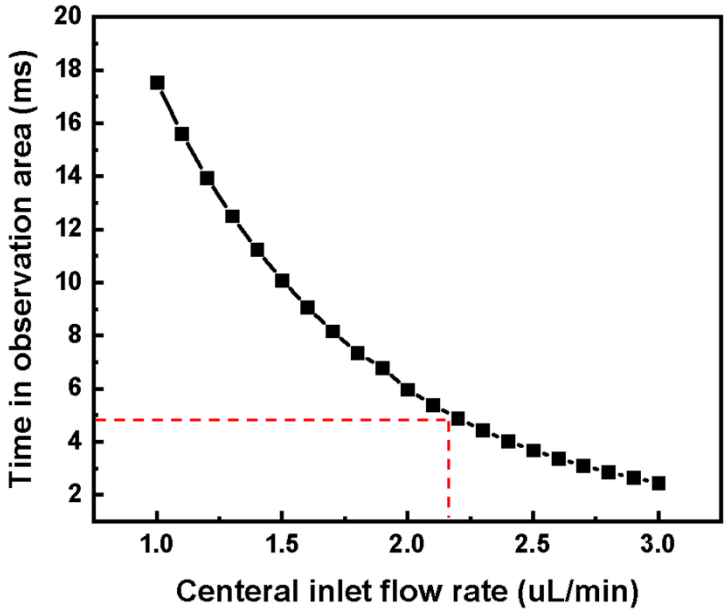

Fig. S5 Time duration in observation area for different flow rate when flow rate ratio between side and center inlets is 5.

5. Hydrodynamic flow focusing

With a flow rate ratio of 1:5 between central and side streams, FEA simulation, green food color dye (center) mixing with water (side) under the microscope, and hyperspectral FT-IR imaging with H_2O (center) and D_2O (side) mixing were performed to visualize the MCFM platform performing hydrodynamic flow focusing.

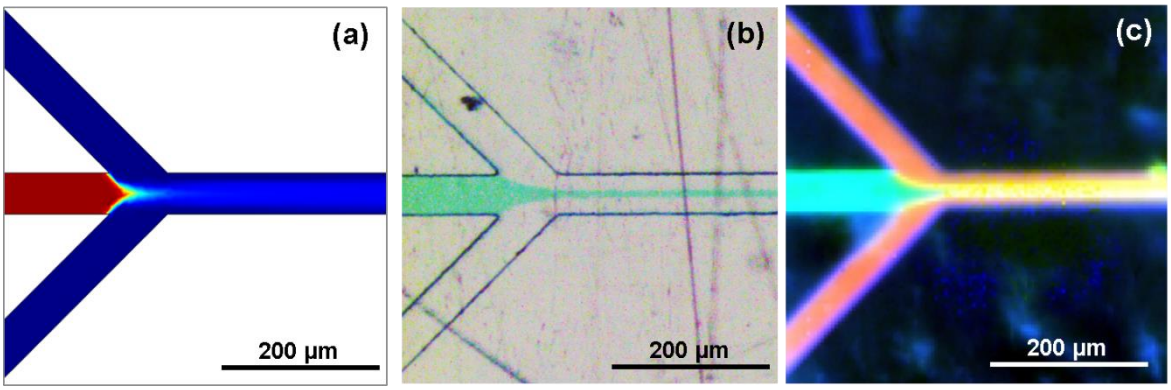

Fig. S6 Hydrodynamic flow focusing from (A) COMSOL simulation, (B) bright field microscope with flow of color dye, and (C) hyperspectral FT-IR imaging with flow of H_2O (green, center) and D_2O (red, side).

6. IR intensity change at 1636 cm^{-1} for H_2O and D_2O mixing experiment

To validate the MCFM platform, a H_2O and D_2O mixing experiment was conducted and observed change in IR absorption of the OH bending mode from both a change in concentration (dilution with D_2O) and due to isotopic substitution (H and D).

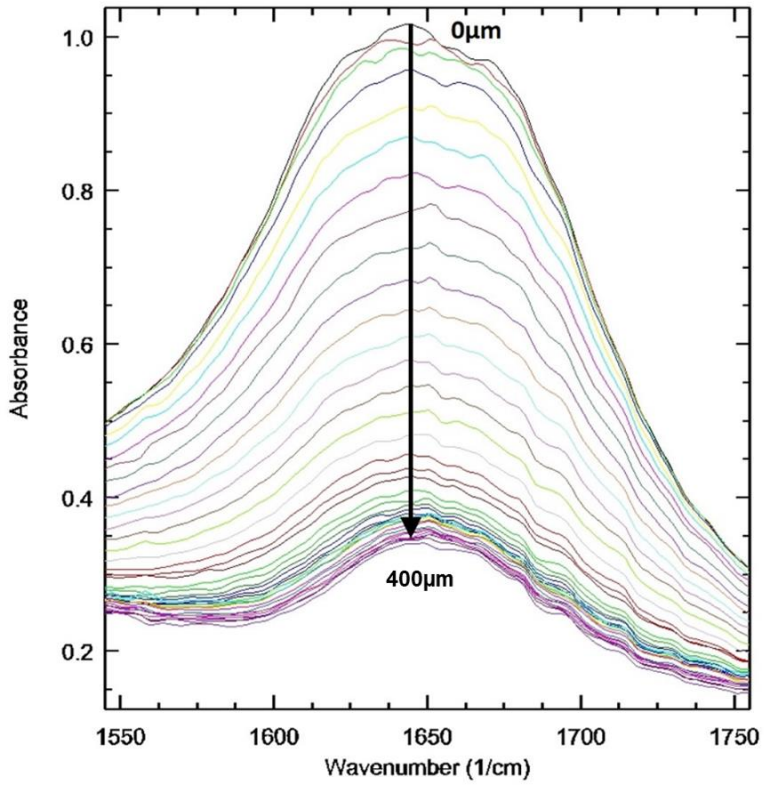

Fig. S7 *Raw spectra data from pixels of the central line in the focused stream along the channel starting from three inlets merging point to 400 µm downstream*. As the mixing started, IR intensity of OH bending decreased.