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Supplementary Information for

A MULTI-PURPOSE MICROFLUIDIC PERFUSION SYSTEM WITH COMBINATORIAL CHOICE OF INPUTS, MIXTURES, GRADIENT PATTERNS, AND FLOW RATES

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Figure S1. Pressurized fluid reservoirs to deliver constant fluid flow. To provide access for pressurization, tubing connectors (for 1/16" inner diameter tubing) were sealed into ports that were drilled in the side of 15-mL tubes. All reservoirs were filled to the same height and connected to a single pressure source in the range from 0.5 to 2 psi (flow rates through the system at these pressures are shown in Fig. 4). Tube caps were also fitted with tubing connectors that on the inside connected to tubing that reached to the bottom of the reservoir. Each line out of the reservoir was passed through a 0.4 µm syringe filter to prevent debris from entering the device. Four colored dyes (red, yellow, blue and green) were split into 4 branches and alternately inserted into the 16 inlets of the PDMS device. An additional reservoir containing water was connected to the rinse line. A 15-mL tube with an open pressurization port was used to collect the flow from the exit port of the device. The device is shown without the pneumatic lines connected.

Video S1: Four movies show the multiplexer operating through sequences of one inlet at a time. The first sequence proceeds from inlets 1 through 16 (left to right) without rinsing between steps. We show views of the multiplexer (MUX) (a) as well as detail of the flow through the central chamber (b). Each inlet was open for 2 seconds at flow rate of 20 μ L/min. Operation of the device in this configuration may not be ideal, as residual compounds in the convergence zone

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may contaminate downstream observations. A rinse step following each inlet (6 s, routed through the chamber bypass not the central chamber) can remove residual chemicals in the convergence zone, as shown in movies of the MUX (c) and chamber (d). We show an additional 2 s rinse through the chamber between each inlet perfusion to differentiate the compounds in the chamber. Only 4 inlets (10-13) are shown in movies (c) and (d) due to the limited acquisition time of our camera (Nikon Coolpix E990). All movies are shown in real time.

Video S2: A chaotic mixing channel was placed downstream of the multiplexer (and upstream of the chamber) to enable complex mixing of inlet combinations. When the mixing channel is bypassed, flow proceeds directly into the chamber, and very little diffusive mixing occurs between the yellow and blue streams (inlets 2 and 4, MUX valves (V) = {10101101}). Passing the flow through the 3-cm long mixing channel increases diffusive mixing between the streams, though it is far from complete. (Positive pressure was applied on the mixing grooves when the grooves are "off" (as is the case for all "closed" valves), which does appear to result in some rotation of the fluid due to the appearance of a green edge on the left of the chamber). Mixing is induced when the PDMS membrane on the bottom of the flow channel is pulled into the staggered-herringbone groove patterns in the pneumatic layer. The movie also shows flow behavior for combinations of 4 inlets (2+4+6+8; V = {10101101}), 2 inlets (1+2; V = {10101011}), and finishes with 2 sets of 4 inlets (1+2+5+6; V = {1011101} and 1+2+3+4; V = {10101111}). Movie is in real time.

Video S3: By changing the flow rate through the central chamber, the amount of diffusive mixing between two or more fluid streams can be precisely tuned using the discrete resistance network. When flow switches from a slow flow rate ($3 \mu L/min$) to a fast flow rate ($36 \mu L/min$), diffusive spreading between the flow streams is greatly reduced. Flow rate was adjusted by switching the resistance valves from a state of all closed {1111} to all open {0000}. Movie is in real time.

Video S4: Laminar flow profiles in the central chamber can be tuned by using the resistance network and valves around the central chamber. With all 16 inlets open and side ports allowing flow out the side of the chamber (though flow out the left side is prevented by a stopper), flow rate through the bottom of the chamber is gradually reduced by running through an increasing sequence of resistor valve combinations. Fluid was driven by 1psi driving pressure on the inlet reservoirs. Movie is in real time.

Video S5: Various functions of the device are demonstrated in a sequence showing different inlet combinations and gradient tuning. Several examples of 2 inlets (1+2, 2+4, 13+15), 4 inlets (1+2+3+4, 2+4+6+8), 8 inlets (1-8, 1+3+5+7+10+12+14+16), and all 16 inlets are demonstrated while the chamber side ports are opened to allow flow out the side of the chamber and resistance valves are switched to change the amount of flow exiting the bottom of the chamber. Movie is in real time.