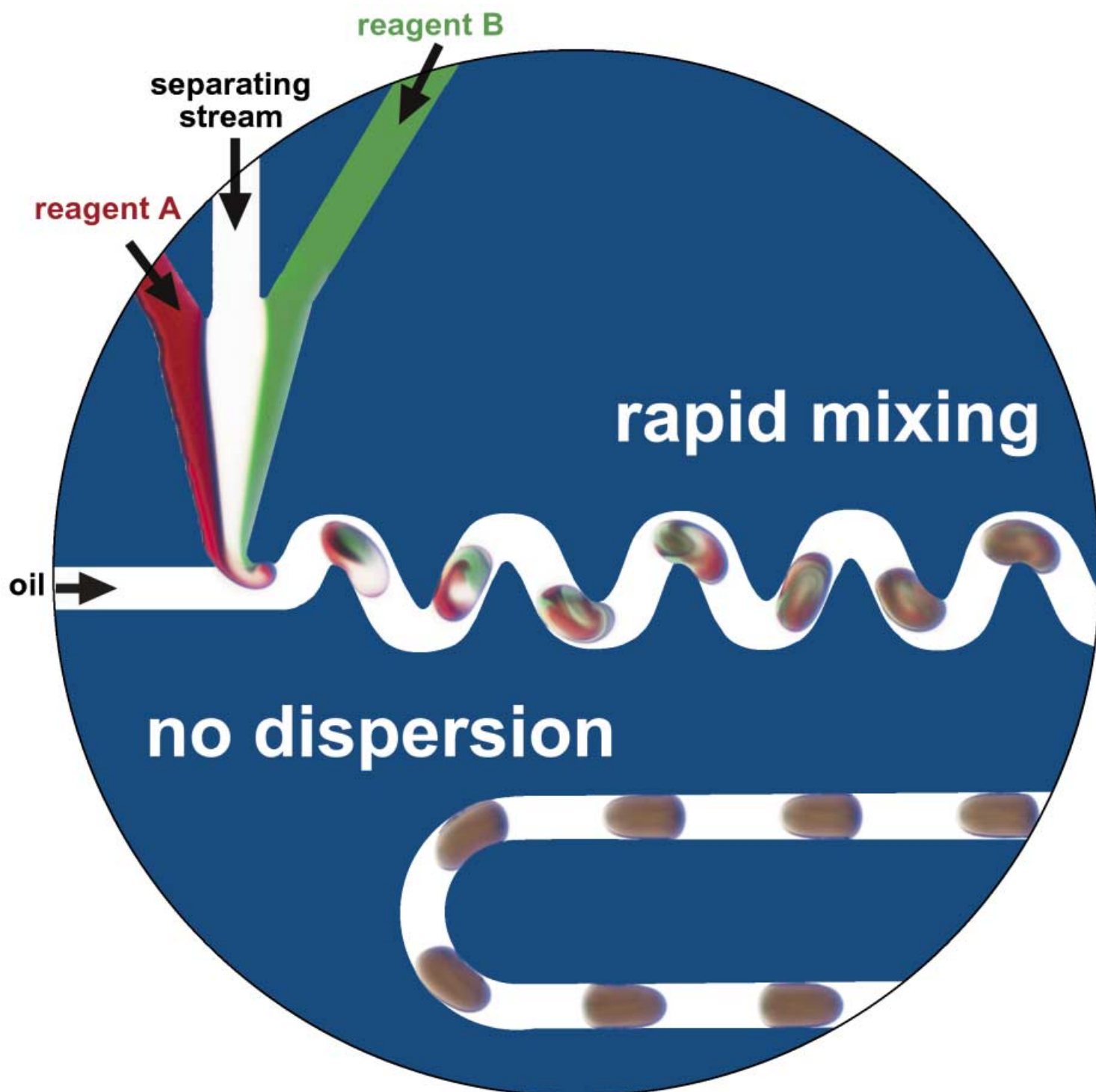


# Communications



Aqueous droplets of 250 pL formed in a microfluidic channel in a continuous flow of a water-immiscible fluid act as microreactors that mix the reagents rapidly and transport them with no dispersion. These droplets may also be used to control chemical reaction networks on millisecond time scale. For more information see the following publication by R. F. Ismagilov et al.

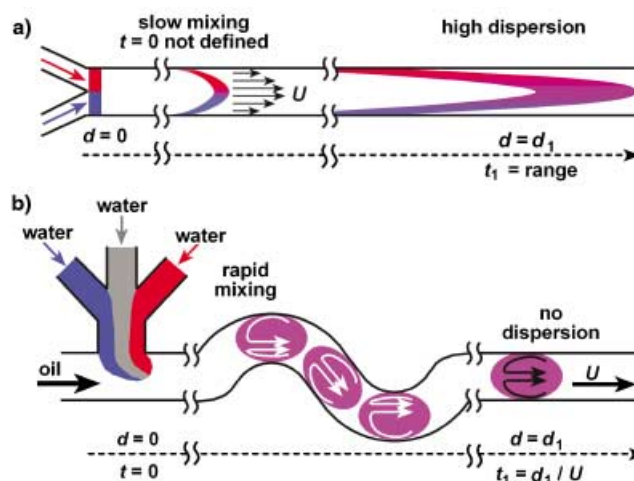
## High-Throughput Measurements

## A Microfluidic System for Controlling Reaction Networks in Time\*\*

Helen Song, Joshua D. Tice, and Rustem F. Ismagilov\*

We present here a microfluidic system that may be used to control networks of many chemical reactions on the milli-second scale. It allows to control when each reaction begins, for how long each reaction evolves before it is separated or combined with other reactions, and when each reaction is analyzed or quenched. The system uses flow of fluids to linearly transform space (length of capillaries) into time (reaction time). For a chemical reaction in an open system this transformation is simple and well known: A solution of reagent A and a solution of reagent B are injected as steady streams into a microfluidic channel at initial point  $d=0$  where the reaction between them begins ( $t=0$ ). As the reaction mixture is transported by the fluid stream at a constant velocity  $U$ , every spatial point  $d$  corresponds to a time point  $t$ , the reaction time, where  $t=d/U$ . If such a system is implemented, interactions of multiple chemical reactions in time could be controlled simply by creating a network of converging and diverging channels carrying reaction mixtures, and varying flow velocities to adjust reaction and interaction times. If the reactions are accompanied by an optical signal (e.g. changes in fluorescence or absorption), time-resolved measurements of the reactions in the entire network could be obtained from a single spatially resolved optical image.

Networks of microfluidic channels<sup>[1–3]</sup> are especially attractive for this distance-to-time transformation because they can be easily fabricated and used to manipulate small volumes of reagents; they are becoming essential for chemical and biological analysis and synthesis.<sup>[1–3]</sup> Flow in microfluidic devices is laminar; it occurs at low values of the Reynolds number,  $Re$  ( $\sim 0.01$ – $100$ ).  $Re$  is defined as  $lU\rho/\mu$ , where  $l$  [m] is the diameter of the capillary,  $U$  [ $\text{m s}^{-1}$ ] the velocity of the flow,  $\rho$  [ $\text{kg m}^{-3}$ ] the density, and  $\mu$  [ $\text{kg m}^{-1} \text{s}^{-1}$ ] the viscosity of the fluid.<sup>[4]</sup> This laminar flow makes it difficult to implement the distance-to-time transformation in microfluidic devices for



**Figure 1.** Schematic comparison of a reaction  $A+B$  conducted in a standard pressure-driven microfluidic system device (a) and in the microfluidic device described here (b). a) Reaction time  $t \neq d/U$ . b) Reaction time  $t=d/U$ . Two aqueous reagents (red, A and blue, B) can form laminar streams separated by a gray "divider" aqueous stream in a microchannel. When the three streams enter the channel with a flowing immiscible fluid, they form droplets (plugs). The reagents come into contact as the contents of the droplets are rapidly mixed. Internal recirculation within plugs flowing through channels of different geometries is shown schematically by arrows.

two reasons (Figure 1 a). First, mixing is slow—two streams injected into a channel flow side-by-side with mixing only by diffusion,<sup>[4–6]</sup> therefore  $d=0$  does not correspond to a well-defined starting point ( $t=0$ ) of the reaction. Significant research efforts have been devoted to solving this problem of slow mixing.<sup>[2,3,7]</sup> Turbulent flows provide both rapid mixing and low dispersion,<sup>[8]</sup> but turbulence occurs at values of  $Re > 2000$  reached in microchannels only at high flow rates ( $\sim 10 \text{ m s}^{-1}$ ). Achieving such flow velocities requires undesirably high sample consumption ( $\sim 1 \text{ mL s}^{-1}$ ) and high pressures. Second, the dispersion of solutes along the channel is large—the flow profile is parabolic, and the reagents are transported at a range of velocities.<sup>[4]</sup> Therefore, a given distance  $d$  corresponds to a range of reaction times  $t=d/U$ .

We developed a simple microfluidic system that overcomes both problems—it transports solutions with rapid mixing and no dispersion (Figure 1 b). This system uses networks of microchannels with rectangular cross sections and hydrophobic surfaces fabricated using rapid prototyping in polydimethylsiloxane (PDMS).<sup>[9]</sup> We controlled the volumetric flow rates through each channel using syringe pumps. Dispersion was eliminated by localizing the reagents within aqueous plugs (droplets large enough to block the channel) separated by a water-immiscible oil. Immiscible fluids have been used to localize reagents in both commercial<sup>[10]</sup> and laboratory<sup>[11–13]</sup> systems. Here we describe methods for forming plugs of multiple solutions of reagents, for using chaotic advection to achieve especially rapid ( $\sim 2 \text{ ms}$ ) mixing within the plugs, and for splitting and merging these plugs in order to create complex microfluidic networks.

To form droplets from two solutions of reagents without bringing the reagents into prior contact we flowed these

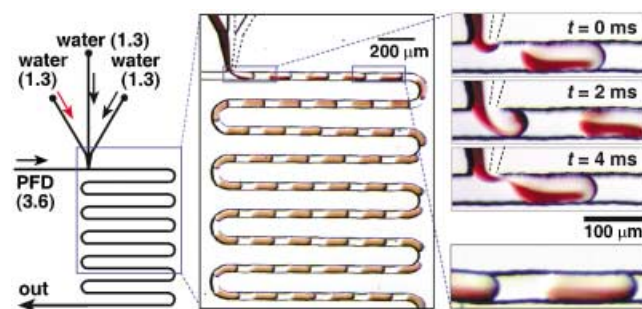
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solutions in a microchannel as two laminar streams,<sup>[14]</sup> and used an inert center stream to separate them. These three streams were continuously injected into a flow of water-immiscible perfluorodecaline (PFD) in the main microchannel, where they spontaneously broke up into streams of plugs (~500 pL) separated and surrounded by PFD (Figure 2). Diffusion through the central stream was too slow for the reagents in the two side streams to interact prior to the formation of a plug. Plugs of three or more reagents could be formed in the same manner. PFD is an attractive choice of carrier fluid because it is inert, immiscible with water and organic solvents, and does not swell PDMS. Emulsions of perfluorocarbons such as PFD are used as blood substitutes in humans during surgeries<sup>[15]</sup> and should be compatible with a range of biological molecules. We controlled the wetting of PDMS by PFD by adding 9% v/v of  $C_6F_{11}C_2H_4OH$  to PFD. Water-in-oil emulsions are known to form in pressure-driven flow in microchannels,<sup>[16,17]</sup> often at high values of the dimensionless capillary number,  $Ca$  ( $Ca = U\mu/\gamma$ ,  $\gamma$  [N m<sup>-1</sup>]: surface tension at the PFD/water interface).<sup>[18]</sup> Our system was operated at low values of  $Ca$  because at high values of  $Ca$ , the shear flow broke up the aqueous phase into small droplets that did not block the channels and were not transported reliably over long distances. Complex behavior in this latter regime has been carefully characterized.<sup>[16]</sup>

A plug moving in a straight channel generates a steady, recirculating flow.<sup>[19]</sup> This flow mixes the fluid within the left and the right (along the direction of the flow) halves of the plug (black arrows in Figure 1b, right), but not between the two halves. Laminar flow is preserved during plug formation that occurs at low  $Re$ ; the reagent solutions end up in the two halves of the plug, and internal recirculation within elongated plugs enhances mixing only moderately (Figure 2).

Chaotic advection accelerates mixing by using unsteady fluid flow to stretch and fold a volume of fluid. Stretching and folding leads to a rapid, exponential decrease of the striation



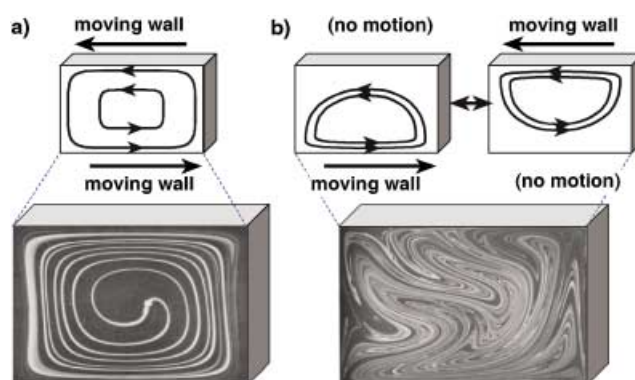
**Figure 2.** Spontaneous formation of uniform plugs out of multiple aqueous streams. Left: Schematic diagram of the microchannel network. Volumetric flow rates for all streams (in  $\mu\text{L min}^{-1}$ ) are given in parenthesis. Middle: Microphotograph (10  $\mu\text{s}$  exposure) of plug formation and transport. Right: Magnified microphotographs (10  $\mu\text{s}$  exposure) of the plug-forming region at different time points. All microchannels had  $50 \times 50 \mu\text{m}^2$  cross sections. Total flow rate in the main channel:  $7.5 \mu\text{L min}^{-1}$  ( $50 \text{ mm s}^{-1}$  average flow velocity);  $Re \sim 2.5$  (water),  $\sim 0.93$  (PFD). PFD here stands for a 10:1 mixture of perfluorodecaline and  $C_6F_{11}C_2H_4OH$ . Red stream: solution of  $[\text{Fe}(\text{SCN})_4]^{3-}$  prepared by mixing  $0.067 \text{ M Fe}(\text{NO}_3)_3$  with  $0.2 \text{ M KSCN}$ ; colorless streams:  $0.2 \text{ M KNO}_3$ .

length—distance over which mixing has to occur by diffusion. At intermediate  $Re$  ( $Re \sim 70$ ), chaotic advection can be generated in three-dimensional microfluidic systems.<sup>[20]</sup> An elegant system used asymmetric ridges on the floor of a microchannel to induce chaotic advection at both low and intermediate values of  $Re$  ( $Re = 0\text{--}100$ ).<sup>[7]</sup> Here we show how the principles of chaotic advection can be applied to mixing inside plugs.

The principle of chaotic advection has been illustrated with flow-cavity experiments.<sup>[21]</sup> In a flow cavity, motion of the top and bottom walls induces flow within a volume of fluid. Simultaneous motion of these walls induces steady flow that mixes solutions slowly (Figure 3a). Time-periodic, alternating motion of the walls induces unsteady, chaotic flow that mixes solutions rapidly (Figure 3b). Crossing of streamlines in this time-periodic flow is a necessary condition for chaos.<sup>[21]</sup>

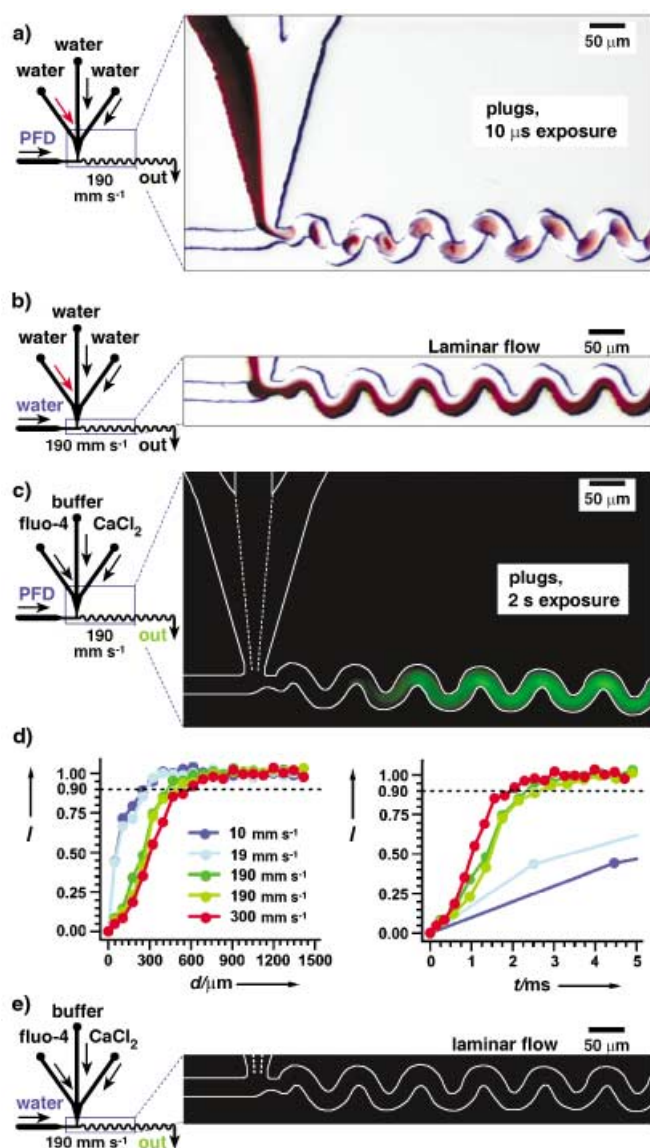
Fluid flow in a flow cavity is similar to fluid flow in a plug moving inside a channel, except for the frame of reference—in a flow cavity walls move relative to the fluid, and in a plug fluid moves relative to the walls. Steady flow in a flow cavity is similar to steady, recirculating flow in plugs moving in a straight channel (black arrows in Figure 1b right). For arbitrary initial conditions, mixing by this steady flow is inefficient (efficiency decreases as  $1/t$ )<sup>[19,21]</sup> because solutes become trapped on the streamlines that do not cross.<sup>[21]</sup> For example, this flow mixes the contents within the left and right halves of the plug, but not between the two halves (Figure 2). The two-dimensional component of this flow responsible for mixing of the solutions can be envisioned as two counter-rotating vortices located in the left and right halves of the plug (white arrows in Figure 1b). In the flow cavity shown in Figure 3 the two vortices are co-rotating.

Pairs of co-<sup>[22–24]</sup> and counter-rotating<sup>[24,25]</sup> vortices both generate chaos in periodic time-dependent flow. We induced such a flow in plugs by replacing a straight channel with a winding channel (Figure 4). A plug moving through a curved part of the winding channel is moving at different velocities relative to the two walls, similar to fluid in a flow cavity of Figure 3b. We have deduced the streamlines shown by the



**Figure 3.** Mixing by steady (a) and time-periodic (b) flows in flow cavities. a, b) Top: Schematic illustration of the flow cavity. The vertical walls are stationary and the horizontal walls move as shown. Bottom: Images (reprinted with permission of Cambridge University Press from ref. [21]) that illustrate the flow patterns. For details, see text.





**Figure 4.** a), b) Microphotographs (10  $\mu$ s exposure) illustrating rapid mixing inside plugs (a) and negligible mixing in a laminar flow (b) moving through winding channels at the same total flow velocity; same solutions as for Figure 2. c) A false-color microphotograph (2 s exposure, individual plugs are invisible) showing time-averaged fluorescence arising from rapid mixing inside plugs of solutions of Fluo-4 (54  $\mu$ M) and  $\text{CaCl}_2$  (70  $\mu$ M) in aqueous sodium morpholine propane-sulfonate buffer (20 mM, pH 7.2); this buffer was also used as the middle aqueous stream. d) Relative normalized intensity ( $I$ ) of fluorescence obtained from images such as shown in (c) as a function of distance (left) traveled by the plugs and of time required to travel that distance (right) at a given flow rate. The total intensity across the width of the channel was measured. Total PFD/water volumetric flow rates (in  $\mu\text{L min}^{-1}$ ) were  $\bullet$  0.6:0.3,  $\bullet$  1.0:0.6,  $\bullet$  12.3:3.7,  $\bullet$  10:6,  $\bullet$  20:6. e) A false-color microphotograph (2 s exposure) of the weak fluorescence arising from negligible mixing in a laminar flow of the solutions used in (c). All channels were 45  $\mu$ m deep; inlet channels were 50  $\mu$ m and winding channels 28  $\mu$ m wide;  $Re \sim 5.3$  (water),  $\sim 2.0$  (PFD).

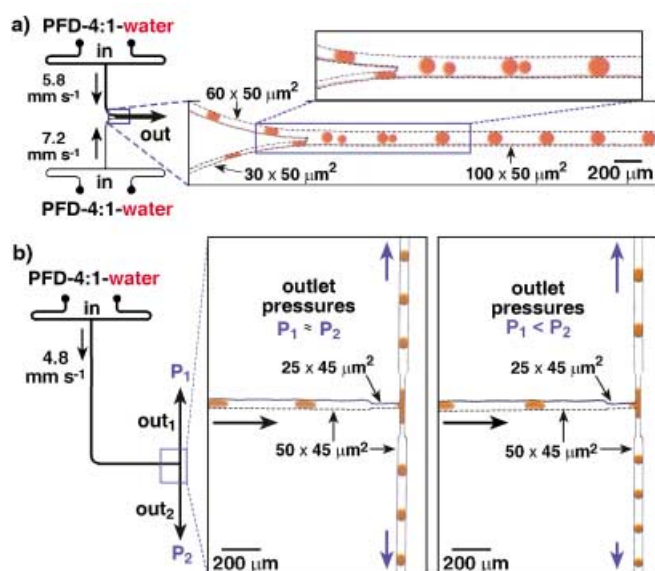
white arrows in Figure 1b by observing asymmetric plugs traveling through curved channels; these streamlines are similar to those shown in Figure 3b. In plugs moving through

winding channels we observed flow patterns that corresponded to very rapid mixing (Figure 4a). These flow patterns were reproducible, and were similar for plugs moving at different speeds, except for some blurring by diffusion at lower speeds. At these low values of  $Re$ , winding channels did not accelerate mixing in laminar flow in the absence of PFD (Figure 4b).

In Figure 4c,d we show that this system can be used to measure the rates of chemical reactions on the millisecond time scale, and that these rates can be used to quantify mixing.<sup>[6]</sup> This was done by measuring the rate of rapid binding ( $k_{\text{on}} = 7.1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ )<sup>[26]</sup> of  $\text{Ca}^{2+}$  ions to the calcium-sensitive dye fluo-4 (Figure 4c). Fluo-4 is weakly fluorescent in the absence of calcium whereas the complex fluo-4- $\text{Ca}^{2+}$  shows strong fluorescence; accordingly, increase in fluorescence corresponds to mixing. At every flow rate we obtained a complete time-resolved reaction profile by acquiring a single spatially resolved long-exposure image of the fluorescence.<sup>[8]</sup> Each image was integrated over two seconds to give the average fluorescence intensity of hundreds of moving plugs ( $\sim 60 \text{ pL}$  in volume) and non-fluorescent PFD, and consumed less than 50 nL of fluo-4 solution. For the laminar flow of these solutions in the absence of PFD only weak fluorescence was observed (Figure 4e) consistent with negligible mixing shown in Figure 4b. Mixing of  $\text{CaCl}_2$  and fluo-4 solutions in the plugs was achieved within a short distance ( $\sim 500 \mu\text{m}$ , Figure 4d), which was not very sensitive to the PFD/water ratio, and was weakly dependent on flow velocity. At higher flow rates it corresponded to mixing in about 2 ms (Figure 4d).

These facts and the flow patterns in Figure 4a are consistent with chaotic mixing, but detailed modeling would be required to identify the chaotic regions within the plugs. Such modeling would have to account for the three-dimensional recirculation within both aqueous plugs and PFD in a given geometry and should suggest the geometry of the winding channels—symmetric or asymmetric, regular or random—required for most efficient mixing.

A key attractive feature of microfluidics is the possibility of creating large fluidic networks.<sup>[27]</sup> To demonstrate how, in principle, a network with complex connectivity could be created with this system we designed methods for combining two streams of plugs into a single stream in a main channel (merging plugs), and for dividing one stream of plugs into two streams (splitting plugs). Merging was rare when the main channel was of the same width as the channels in which the plugs formed as plugs merged only if they entered the main channel simultaneously (in phase). Plugs that entered out of phase continued through the main channel separated by PFD because they all moved at the same constant velocity. By contrast, merging was achieved in a wide main channel, even for streams of plugs that were out of phase (Figure 5a). We formed small and large plugs in 30- $\mu\text{m}$  and 60- $\mu\text{m}$  wide channels, respectively. These plugs did not block the expanded 100- $\mu\text{m}$  wide main channel and could move at two different velocities until they coalesced.<sup>[28]</sup> Small droplets moved more slowly than large droplets;<sup>[16]</sup> this effect is well known and is the basis of hydrodynamic chromatography.<sup>[29]</sup> Sufficiently large fluctuations in the relative flow rates in the two channels disrupted merging, but more than 95 % of the plugs merged



**Figure 5.** a) Spontaneous merging of pairs of plugs into single plugs. Left: schematic diagram of the microfluidic network; right: microphotograph of two streams of plugs merging in a main microchannel. b) Spontaneous splitting of plugs at a branching point in a microchannel. Left: schematic diagram of the microchannel network and the experimental conditions used. Middle: microphotograph showing splitting of a stream of plugs into plugs of approximately one-half the volume of the initial plugs. The outlet pressures were equal for the two channels. Right: asymmetric splitting of both aqueous and PFD plugs occurred when hydrostatic pressure of about 6 kPa was applied to the second outlet. a), b) Volumetric PFD/water flow rate ratios, flow velocities, and cross-sectional dimensions of the channels (width  $\times$  height) are also shown.

correctly when flows were simply driven with pressure of compressed air from a house line, regulated manually.

Reliable splitting of plugs at branching points of microchannels was achieved by constricting the channels at the branching points. When the channels were not constricted, short rounded plugs often showed a complicated behavior dominated by the surface tension at the PFD/water interfaces. In constricted channels the plugs became long and narrow and behaved simply like laminar flows subjected to pressure gradients (Figure 5b). The size of the split plugs was proportional to the relative flow rates in the two outlet channels; we controlled these flow rates by varying the relative hydrostatic pressures at the two outlets. Splitting and subsequent merging of plugs could become useful for high-throughput parallel measurements. These methods of merging and splitting rely on a simple control of widths of channels and should become especially useful in combination with advanced methods of pumping.<sup>[30]</sup>

Overall, the phenomena described here allow us to construct a microfluidic system that linearly converts distance into time by achieving millisecond mixing and transport with no dispersion. We believe that it will be useful for both chemical analysis and synthesis, and for studies of complex reaction networks.<sup>[31]</sup> The system is easy to fabricate and operate, has no active or moving components, and can be controlled simply by varying the rates of fluid flow. The

uncertainty of the distance-to-time transformation is limited by the mixing distance. Both short (ms) and long (min) time scales can be accessed in the same microfluidic device simply by varying the flow velocity. Sub-millisecond time scales should be accessible by decreasing the size of the channels, and by increasing the flow rate (as long as the capillary number remains small). In a serpentine microfluidic network an entire time course of a chemical reaction can be obtained with millisecond resolution by analyzing fluorescence from a single image. Because the flows are steady this image does not have to be time-resolved<sup>[8]</sup> and can be obtained with long exposures of a CCD or even a photographic camera. This system consumes samples at a rate about 10000 times lower ( $\mu\text{L min}^{-1}$ ) than devices that rely on turbulence for mixing ( $\text{mL s}^{-1}$ ). It may serve a broad community of chemists, biochemists, and biophysicists as an inexpensive complement to stop-flow instruments. In addition to a planar, trivial to fabricate, disposable plastic chip it only requires equipment already present in most laboratories: a source of pressure, such as a syringe pump or a cylinder of compressed gas, and a microscope with a CCD camera. We believe that this system for conducting reactions in sub-nanoliter plugs will also become valuable in traditional areas of microfluidics where miniaturization and speed are important—for example, high-throughput screening, combinatorial synthesis, analysis, and diagnostics—both as a self-contained platform, and in combination with the existing technologies, especially those using flows of immiscible fluids.<sup>[32,33]</sup>

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