

oblique angles to the axis of flow. The wells may be of various depths, however, for a given set of reagents, flowrates, and channel geometries, there may be an optimum depth of a well wherein an increased depth may not increase the mixing effectiveness.

[0072] Fabrication and operation of the passive micromixing apparatus of the present invention is simplified over approaches that require splitting the fluid flow into multiple smaller channels and then recombining. By contrast, mixing in accordance with the present invention is the result of transverse flow and fluid folding in a single channel, thereby permitting effective mixing of confluent streams with high flow rates.

[0073] Electroosmotic flow is a surface driven mechanism that may be enhanced to change the performance of the present invention. For example, increasing the electroosmotic mobility of selective surfaces, such as of the wells, has been shown to increase the effectiveness of the mixer.

[0074] The manufacturing process and equipment used in the experiments referenced in this specification are herein defined.

[0075] Reagents and Materials. Laser Grade Rhodamine B was used as supplied by Across Organics (Belgium) and dissolved in 20 mM, pH 9.4 carbonate buffer to a final concentration of 0.11 mM Rhodamine B. The buffer solution was made using deionized water from a Millipore Milli-Q system (Bedford, Mass.), and was filtered before use with a syringe filter (pore size 0.22 μm).

[0076] Microchannels were made using polycarbonate sheet (PC; Lexan, GE Co., Mt. Vernon, Ind.). Poly(ethylene terephthalate glycol) (PETG; Vivak, DMS Engineering Plastic Products, Sheffield, Mass.) was used to cover and seal the microchannel substrate. The glass transition temperature of PC and PETG are approximately 150° C. and 81° C., respectively. Polycarbonate was chosen as the substrate material because it has a high absorption cross section to 248 nm light (the wavelength of the excimer laser), therefore ablated structures have minimal surface roughness (<5 nm). PETG was chosen to seal the microchannels because its glass transition temperature is well below that of PC. Therefore, thermal sealing can be performed at a temperature that does not cause distortion of the PC microchannel.

[0077] Hot Imprinting Method. Prior to imprinting, the PC substrate was blown clean with ionized air. Channels were hot imprinted in the substrate material using a silicon stamp with a trapezoidal-shaped raised T-channel. The PC was placed over the silicon stamp, the two items were then placed between two aluminum heating blocks, and then the temperature was raised to 155° C. Next, the assembly was placed in a hydraulic press and a pressure of 13.8 MPa (2000 psi) was applied for 1.5 hours. The imprinted substrate was then removed from the template and allowed to cool to room temperature. Channel dimensions were measured by optical profilometry.

[0078] Laser Ablation Method. A 248 nm excimer laser system (LMT-4000, Potomac Photonics, Inc., Lanham, Md.) was used to ablate microstructures within the preformed PC microchannel. The excimer laser system, FIG. 18, contains a laser light source 1, a round aperture (200 μm diameter) 2 for delimiting the size and shape of the beam 3, a focusing lens (10 \times compound) 4, a visible light source 5, a CCD camera to image the ablation process 6, and a controllable X-Y stage 7 with a vacuum chuck 8 to hold the substrate 9 in place. Also, a nozzle 10 was present to sweep nitrogen over the substrate 9 during processing, and a vacuum nozzle 11 was located on

the opposite side of the stage to remove debris. For the experiments conducted here, the size-delimiting aperture was chosen such that the ablated features would be smaller than the dimensions of the channel. Also, the X-Y stage was moved linearly at a rate of 1 mm/s, and the ablated wells were at a 45° angle relative to the axis of the main channel. The average power level per pulse was set to 2.04 μJ \pm 0.14 μJ . The frequency of pulses was set to 200 Hz, with a constant pulse width of 7 ns. The light after being focused exposed a circular area of 1.90 $\times 10^{-6}$ cm^2 .

[0079] Measuring Well Depth and Profile. The depth of the ablated wells was measured by cutting the substrate with a microtome (Microm HM335 E, Walldorf, Germany) either perpendicular to the axis of the outlet channel or parallel to the slanted wells. The substrate was cut so that the edge of the substrate was within a few microns of the wells. The wells were then imaged and measured using white light microscopy.

[0080] Microchannel Sealing Procedure. The pre-formed microchannels were covered and thermally sealed with a flat piece of PETG (referred to as the 'lid' throughout the rest of the text) of similar dimensions to the PC. Prior to bonding, the lid and the channel were cleaned with compressed nitrogen gas. The lid was then placed on top of the channel, and the two pieces were clamped together between microscope glass slides and bonded by heating in a circulating air oven at 90.0° C. \pm 0.5° C. for 13 minutes. It is important to keep the time and temperature as low as possible in the sealing process to avoid physical alteration of the microchannel.

[0081] For the electroosmotic flow studies, 3 mm diameter circular holes in the lid provided access to the channels and served as fluid reservoirs. For the pressure driven flow studies, 0.8 mm diameter circular holes in the lid, located at the ends of each inlet channel, provided access to insert a section of hollow stainless-steel tubing. A 3 mm diameter hole in the lid at the end of the outlet channel served as a waste reservoir. For each experiment, the channel arms were fixed to a length of 8 mm.

[0082] Flow Image Acquisition. Fluorescence imaging of the rhodamine dye was performed using a research fluorescence microscope equipped with a 10 \times objective, a mercury arc lamp, a rhodamine filter set, and a video camera (COHU, San Diego, Calif.). Digital images were acquired using Scion Image™ software and a Scion LG-3 frame grabber (Scion, Inc., Frederick, Md.). For each experiment, images were captured every 1/60th of a second over a duration of 0.67 s, averaged, then recorded.

[0083] Experimental Set-up. To image the mixing under electroosmotic flow, the microchannels were initially filled with the carbonate buffer solution. Then, an equal amount (typically 40 μL) of buffer was placed in one inlet channel reservoir and in the outlet channel reservoir, while the second inlet reservoir was filled with the rhodamine-labeled buffer. Platinum electrodes were then placed in contact with the solution in the reservoirs such that the two inlet reservoirs were fixed to ground and the potential was applied to the outlet channel reservoir. The microchannel was placed beneath the fluorescence microscope described in the previous section, and images were acquired at several different applied voltages (0 to -1750V), beginning with zero applied voltage to verify that there was minimal flow resulting from hydrostatic pressure. The current through the microchannel was determined by measuring the voltage drop across a 100 k Ω resistor (typically less than 1/1000 the resistance of the