Three-Dimensional Hydrodynamic Focusing with a Two-Layer Microfluidic Device: Controlling the Vertical Position of the Focused Stream

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Abstract:
A microfluidic manifold has been designed, fabricated, and tested which hydrodynamically focuses a sample into the center of a microchannel and provides control over the vertical position of the focused sample via the flow-rates of the focusing fluids [1]. To characterize the focusing action, a mixing experiment was performed in which the sample fluid and focusing fluid contained spectrally unique fluorescent dyes. By sweeping the ratio of the rate of the top focusing fluid to the rate of the bottom focusing fluid, the focused sample was positioned first near the top of the microchannel and then translated downward in steps to the bottom of the microchannel.

Introduction:
Vertical steering of a centrally focused fluid stream in a planar microfluidic device is useful for developing portable optical biosensors. Optical biosensors historically have relied on mechanical micropositioning equipment to align the flow-cell with the optical detection system. In the development of microfabricated flow cytometers, several groups have taken steps to eliminate micropositioning equipment by imbedding integrated optics directly into the flow-cell [2-4]. In such static arrangements where the optical components remain permanently fixed in their positions, the ability to control the vertical position of the particle stream by adjusting the flow-rates of the focusing fluids provides an attractive method for tuning the alignment between the sample and the optical detection system.

Figure 1: ANSYS simulations and confocal micrographs showing the effect of adjusting the flow-rate of the upper focusing fluids relative to the flow-rate of lower focusing fluids.
The first microfluidic hydrodynamic focusing devices focused a sample from two sides into a thin columnar stream [5]. Initial designs for focusing not only from the sides but also from above and below involved several fabrication steps with as many as five-layers of manifold housing [6]. Recent efforts have focused on fabrication simplicity. Manifolds capable of out-of-plane focusing have been constructed from two patterned microfluidic layers [7-9]; these devices function by overlapping the channels of the first layer with those of the second layer at junctions where the sample is impinged by fluid from the adjoining layer.

Here, we describe a two-layer manifold following a design similar in concept to Chang et al. 2007 but containing only two-stages of focusing instead of three-stages. This advance reduces the number of required fluid inputs for three-dimensional focusing.

**Description of the Manifold:**

The manifold was constructed from two complementary pieces of poly-dimethyl siloxane (PDMS). The dimensions of the manifold were h = 125 µm, w₁ = 125 µm, w₂ = 50 µm, and w₃ = 50 µm. The sample was applied to the first input at volumetric rate \( U_1 \), and focusing fluid was applied to the focusing inputs at rates \( U_2 \) and \( U_3 \). The flow-cell contained a total of five fluid inputs. Focusing fluid was applied by a symmetric pair of fluid inputs from both sides of the main microchannel at each focusing stage; therefore, the total flow-rate was \( U_0 = U_1 + 2U_2 + 2U_3 \). For the duration of the experiment, the flow-cell was operated under continuous flow conditions at Reynolds number \( Re = 2.72 \), which corresponds to a total volumetric flow-rate of \( U_0 = 20.4 \mu L/min \) (2.20-cm/s). The ratio of sample fluid to total fluid was fixed at \( U_1 / U_0 = 1/10 \) (Figure 1).

**Results:**

The distributions of fluids at the output of the manifold were imaged by scanning through the depth of the microchannel using a confocal microscope. The focusing ratio, \( U_3 / U_2 \), was varied, and several images were obtained. For small values of \( U_3 / U_2 \), the sample was positioned near the top of the microchannel as shown in Figure 1a, whereas for large values of \( U_3 / U_2 \), the sample was positioned near the bottom of the microchannel as shown in Figure 1e. In the case of \( U_3 / U_2 = 1.5 \), the sample fluid was sheathed on all sides and was positioned near the geometric center of the microchannel as shown in Figure 1c.

**References:**


