A Non Coaxial Sheath Flow Device for Micrometer Sample Stream Profiles

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In this paper we present a novel non coaxial sheath flow device with channel dimensions of 50 µm in square at the focusing region. The device is suitable for one-by-one cell or particle detection near the channel bottom, in for example a Coulter Counter or for optical near field detection. The device comprises a silicon-glass sandwich with SU-8 resist in between. Analyses of the adaptable sample flow are carried out where the focusing limitation and its profile variations are shown using confocal microscope measurements. A comparison of the sample flow height inside the channel and its relative fluorescence intensity is highlighted.

Introduction

In a non coaxial sheath flow device a sample flow is hydrodynamically focused between three sheath flows. At the fourth side the sample flow is moving along the channel bottom, where sensors for characterizing small particles, cells or biological molecules are integrated. The position, the size and the profile of the sample flow can be dynamically adapted, as shown in [1]. This hydrodynamic focusing technique is an attractive way to achieve a small sample flow in microfluidic devices which prevents channel clogging, because of the relatively large channel size. Hydrodynamic focusing is typically used in flow cytometry, e.g. FACS (fluorescence activated cell sorting) and Coulter Counter. A review of different flow cytometer systems is listed in [2], where optical and impedance analysis has been described in the field of disease diagnostics, cell/molecular biology and genetics.

For characterizing particles or cells in the dimensions of a few microns or smaller a sample stream in the same range is needed. Experiments on focusing limitation of the sample flow in a non coaxial sheath flow device have been presented in [3] where a stream of 10 µm in width was achieved by a channel width of 160 µm. One way to achieve smaller sample diameters is to reduce the dimensions of the microfluidic device. In this paper a novel down scaled non coaxial sheath flow device is described, which allows a sample flow in the dimension of a few micrometers to analyze particles or cells in the same range. The focusing limitation of the sample flow of this device is shown. Additionally, a comparison of the sample flow height inside the channel and its fluorescence intensity is highlighted.

Device Description

Fabrication and Design

The non coaxial sheath flow device is manufactured with a planar microfabrication process using photolithography, combined with anisotropic silicon etching, photoresist structuring and adhesive low temperature bonding technique. The whole fabrication and clean room logistics were carried out on 100 mm wafers. The chip consists of a

silicon-glass sandwich with SU-8 resist in between. In Fig. 1 a photograph of the fabricated chip is depicted. The access holes through the 360 µm thick silicon wafer were anisotropically wet etched with a KOH water solution. The channel structure was defined by the 50 µm thick lithographically processed epoxy resist SU-8. As a cover plate an optical transparent PYREX glass wafer (200 µm thick) was used. The silicon wafer and the glass wafer were thermally bonded at 150°C [4]. Finally the bonded wafers were diced with a conventional wafer saw.

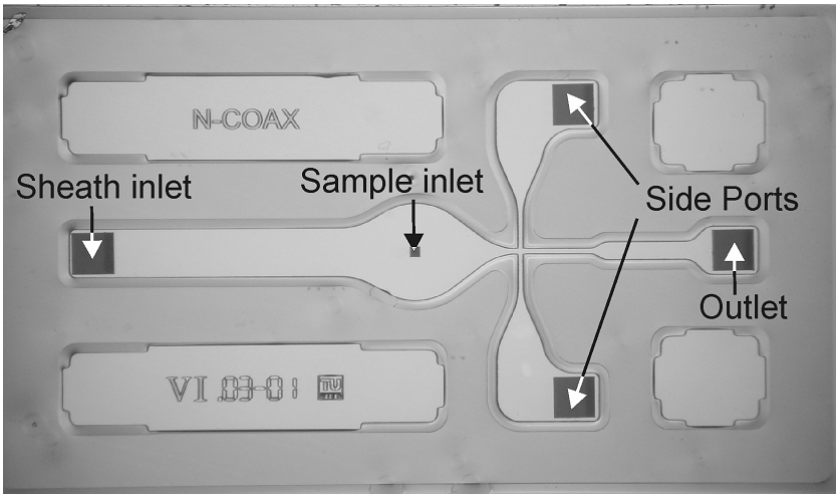

Fig. 1: Photograph of the non coaxial sheath flow device (chip dimensions: 6 x 9 mm^2); Sheath inlet, side port inlets, and outlet: 330 x 330 μm^2; sample inlet: 80 x 80 μm^2; channel dimensions after the focusing section: 50 x 50 μm^2.

At the sample inlet the sample is injected, which flows along the channel bottom. The sample is first focused by the taper. By controlling the flow rates of the sheath inlet and the side ports relatively to the sample flow rate the sample profile and its size can be varied.

Measurement setup

For experiments the non coaxial sheath flow device is fixed on a custom made holder to achieve fluidic connections to syringes. Syringe pumps (kdScientific model 200 series) define the flow rates at the different inlets. A diluted fluorescent dye (acridine orange) is used for the sample flow and the sheath flow is deionized water. During the experiments the chip is positioned in a confocal laser scanning microscope (Confocal C1 TE300, Nikon). This microscope not only allows to capture vertical images of the device with a digital still camera (D100, Nikon) for quantitative analysis but it also permits to measure the precise profile of the sample flow inside the channel. These two analysis techniques allow comparing the relative fluorescence intensity of the sample flow to the real sample height inside the channel.

Experiments

Focusing Limitation

The width of the sample flow is optically analyzed by taking a photo with a digital reflex camera. The concentration of the emitted fluorescent dye is evaluated. The width of the sample flow is defined as the width of the sample at 50 % of the maximum dye inten-

sity. The measured focusing limitation of this device is a 2.5 µm wide sample flow at a channel width of 50 µm. The flow rate at the inlet ports are held at 2 µl/min (sheath inlet), 0.1 µl/min (sample inlet) and 30 µl/min (side ports). The focusing limitation of this device is depicted in Fig. 2. The image presents the emitted green color of the fluorescent dye (wavelength of 520 nm). The reason of the focusing limitation is the low velocity of the pressure driven parabolic flow near the channel bottom.

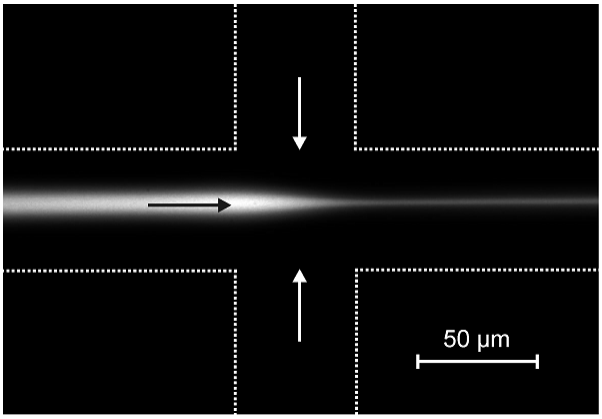

Fig. 2: Focusing limit of the sample flow in a non coaxial device (The edges of the channels are outlined with a dotted line for reference and the arrows indicate the flow direction): The smallest width of the sample flow amounts to 2.5 µm.

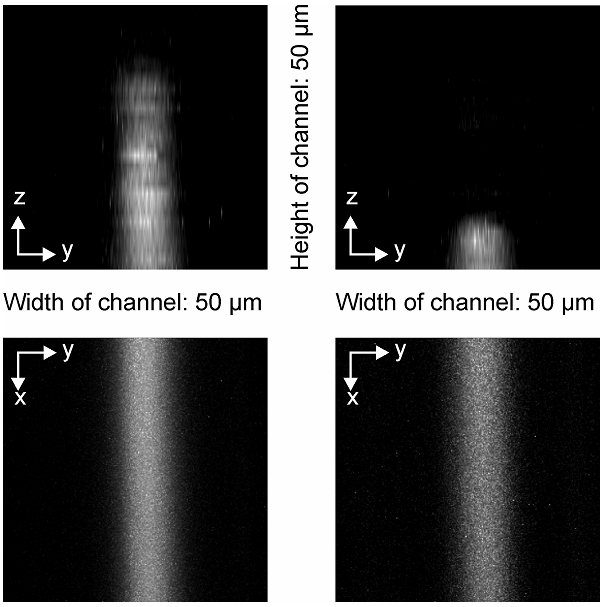

Fig. 3: Top: Confocal images of the sample flow profile inside the channel; Bottom: Photos of the fluorescence concentration of these sample flows.

Confocal Images vs. Intensity Measurements

Measurements with the confocal laser scanning mode of the microscope are constituted in order to get the precise profile of the sample flow inside the channel. In Fig. 3 top left and top right measured profiles of the sample flow are depicted. The flow rate at

the sheath inlet is set to 10 µl/min and at the side ports there is no flow. At the top left and bottom left images the sample flow rate is held at 2 µl/min (sample height of 35 µm) and at the top right and bottom right at 0.05 µl/min (sample height of 8 µm). The two bottom images of Fig. 3 show a vertical section of the whole channel.

The height of the sample flow can be determined by comparing the intensity of the sample flow with the intensity of a sample flow reaching the channel ceiling. This assumption is valid while the green color of the camera is not in saturation. For this experiment, the flow rate of the sheath flow is constantly set to 10 µl/min and on the side ports there is no flow. The flow rate of the sample flow is varied: 0.05 µl/min, 0.1 µl/min, 0.5 µl/min, 1 µl/min, and 2 µl/min. The fluorescence concentration increases by increasing the flow rate of the sample flow. The fluorescence concentration relatively to the reference curve allows determining the sample height inside the channel.

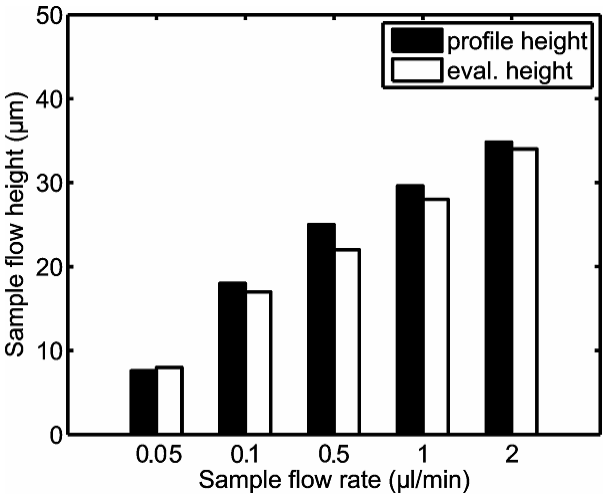

Fig. 4: Comparison of the measured sample profile height (black bars) in the channel and the evaluated sample flow height (white bars) over the fluorescence intensity. Flow rates: 10 µl/min (sheath inlet), 0 µl/min (side ports).

Fig. 4 compares the sample height measured by the confocal microscope and the calculated sample height found from the relative fluorescence intensity. The confocal images were performed at 0.5 µm per *z*-sectioning step over the 50 µm channel height. The results are in good agreement, so it is possible to determine the height of the sample stream using intensity measurements inside a micro channel. Taking top viewed fluorescence photos of the sample stream inside the micro channel the width and the height of the stream can be determined over color intensity. The precise shape of the sample flow can still not be determined.

Conclusion

In this paper, investigations of a novel scaled-down non coaxial sheath flow device with a channel cross-section at the focusing area of 50 x 50 µm^2 have been shown. The experimental determined focusing limitation of the sample flow is highlighted and amounts to 2.5 µm in width. This micro-scaled sample flow can be used to characterize particles or cells in the same order of magnitude. To get information of the sample profile inside the micro channel confocal laser scanning microscope measurements were constituted. The confocal measured heights of the sample profile are compared with the sample flow heights calculated from the fluorescence intensity of the sample. The results show good agreement. This analysis shows that without using extensive and

high priced confocal laser scanning measurement equipment the sample height can be estimated by taking top viewed fluorescence photos of the sample stream inside the micro channel. The sample height will be determined over the color concentration, although the shape of the sample flow is still unknown.

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