Flow Cytofluorometry with a Soft Elastomer-on-Silicon Nano Photonic Device for High-Speed Fluorescence Multi-Spectrum Acquisition

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Abstract:

Flow cytofluorometry is an invaluable tool in separating and analyzing biological samples. Applications of this technology include medicine, biology, and Homeland Security. This project fabricated a microfluidics flow cell of polydimethylsiloxane (PDMS) to demonstrate on-chip flow cytometry. The fabricated device allowed for detection of fluorescent beads and discrimination of their relative sizes as they passed the interrogation zone. The use of this flow cell in conjunction with a nano photonic microelectromechanical systems (MEMS) device will lead to real-time measurement of fluorescence spectra.

Introduction:

Traditional multispectral flow cytometry measurements use multiple photomultiplier tubes (PMT) to detect photons of varying wavelengths from fluorescent samples. This approach is costly, requiring complex, bulky optics. Steven Truxal, Yi-Chung Tung, and Dr. Katsuo Kurabayashi have developed a novel MEMS device that uses polydimethylsiloxane (PDMS) as a transparent, flexible nano-grating for diffracting multispectrum light and directing it onto a single photomultiplier tube. Coupled with a well-designed microfluidic flow chamber, the device can be used to realize an on-chip multispectral flow cytometry system.

The object of this project was to fabricate a flow cytometer to generate emission light from fluorescent beads passing through flow cell microchannels as part of our aimed system. This will show whether emission intensities are strong enough for fluorescence spectra measurements.

Fabrication:

The flow cell consisted of a PDMS slab with micro patterns that acted as inflow, outflow, and microchannels. To make the flow cell mold a 20 μm photoresist layer of SU-8 10 was spun onto a 4 inch silicon wafer. The wafer was then exposed at 11 mW/cm^2 for 20 seconds with 365 nm light under a pre-existing lithography mask. The wafer was then developed in SU-8 developer until the high aspect ration "hills" were revealed (see step 2 in Figure 1).

Next, PDMS was mixed using the Sylgard 184 silicone elastomer kit (Dow Corning Corporation, 10:1 base-curing agent ratio). The viscous PDMS was placed in a vacuum for 30 minutes to remove any bubbles. The mold was placed in a handmade, aluminum foil cup; PDMS was poured over the mold, and then cured at 150°C for 10 minutes. The solidified PDMS was then peeled from the mold. The PDMS now had 20 μm deep patterns that act as microfluidics channels.

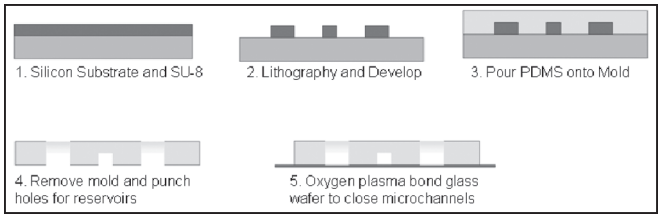

Figure 1: Microfluidics flow cell fabrication.

The mold consisted of several different patterned microfluidic devices. Each was cut from the PDMS to create individual cytometers. Using a 2 mm biopsy punch, holes were punched through the PDMS to create inflow and outflow openings. The PDMS devices were then treated with a O_2 plasma along with a thin glass wafer (250 mT, 80 W, 45 seconds, 17% 2 O, March Asher), then bonded. The flow openings and microchannels were sealed by the glass, leaving openings at the top of the device for inflow and outflow tubing (see Figure 2). Flow cells with channels of 340 μm in width, and 20 μm and 70 μm in depth were fabricated.

Experiment Setup:

The performance of the fabricated microfluidic flow chamber was characterized by observing individual fluorescent beads passing through its microchannel. By measuring the emission light intensity of fluorescent beads, their relative size can be distinguished. Fluorescent beads of diameters 6.5, 31, and

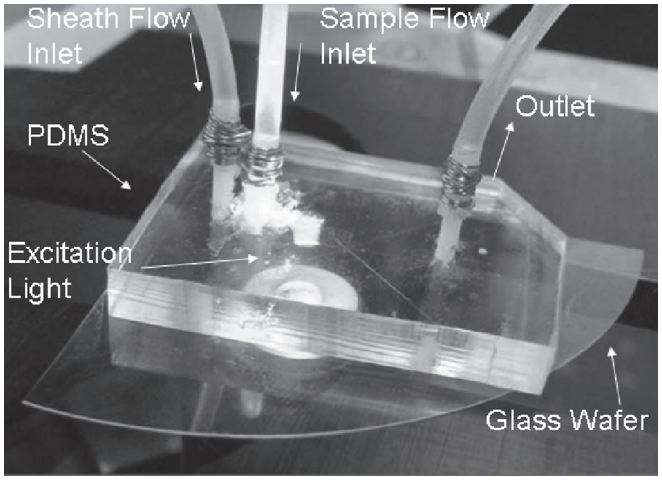

Figure 2: Flow cell excited on microscope stage.

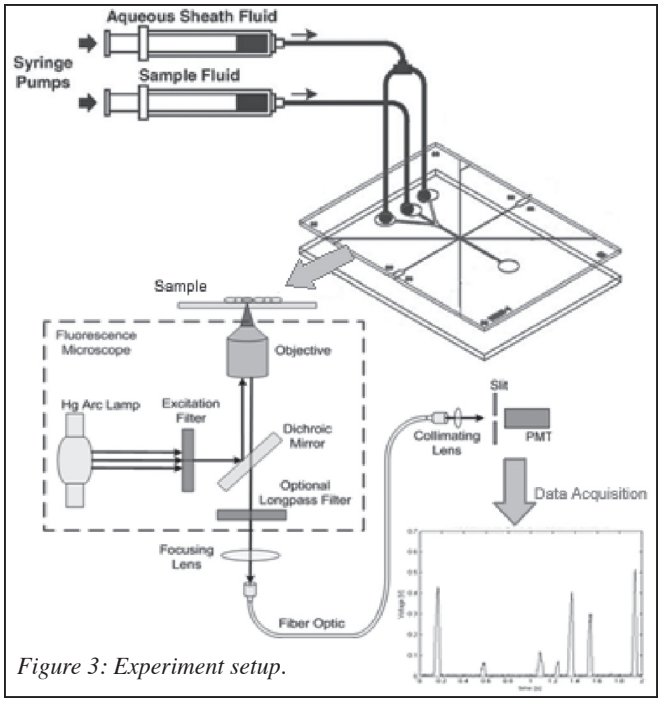

Figure 3: Experiment setup.

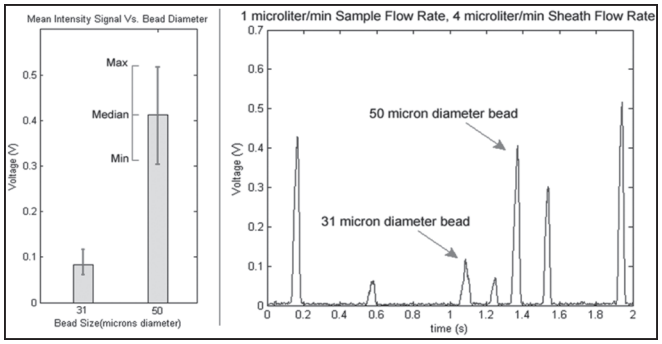

Figure 4: Flow observations.

50 μm were driven through the microchannels. As the beads flowed through the microchannel, they could be observed optically via microscope or the emission light could be directed by fiber optic cable to the PMT for data acquisition (see Figure 3).

Results and Discussion:

The measured intensities provided information on several characteristics of the bead samples and flow cell. The emission intensities could be compared, as in Figure 3, and their relative sizes of the beads could be determined. The mean intensities for 31 μm beads were about 0.9 V and 0.4 V for 50 μm beads. We also determined that 6.5 μm beads require an oil emersion lens to be detected out of the ambient noise in our system.

Conclusion:

We demonstrated simple flow cytometry analysis can be done using MEMS technology. The size of different particles can be determined by their emission light intensity. The measurement error may be reduced by using a two dimensional sheathing flow to direct fluorescent particles closer to the objective lens. Emission light intensity passing through the nano photonic device may be reduced to 10%. Experiments show the emission light intensities are high enough for fluorescence spectroscopy measurements.

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References:

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