Micro-fluidic Chip for Flow Cytometry

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ABSTRACT

This lab course is intended to give students hands-on experience with microfabrication. The project is to make a micro-fluidic chip for flow cytometry. From class, students will learn how to do photolithography, silicon bulk-etching, oxidation, metallization, lift-off, and glass to wafer bonding. After chip being completed, it will be tested and student can see how the hydrodynamic focusing and electrokinetic focusing can significantly enhance the mass detection efficiency for laser induced fluorescence (LIF) based molecular sensing by concentrating fluorescence labeled molecules in a tiny probe region.

INTRODUCTION

The goal of this class is to enhance the detection efficiency for laser-induced fluorescence (LIF) based molecular sensing by using on a micro-fluidic chip. The conceptual layout of such a micro-fluidic chip is shown below:

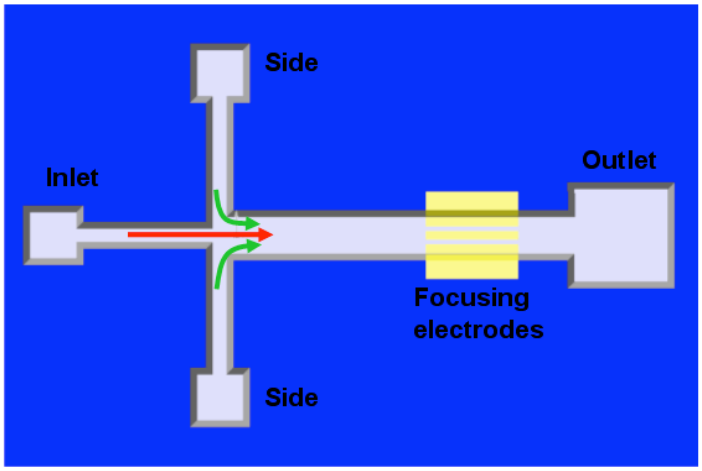


Figure 1. Micro-fluidic processing chip [1, 2, 3]

The detection and identification of molecules and cells with high sensitivity is required in many fields, such as molecular biology, medical diagnostics,

drug delivery, and forensic analysis. The advanced development of laser induced fluorescence (LIF) and confocal microscopy techniques have made possible the detection on the single molecule level. In the LIF based detection technique, molecules are detected in a limited focal volume of a laser beam. Typically the probe region can be defined smaller than 100fL. Background radiation caused by Rayleigh and Raman scattering of the laser beam by solvents is minimized due to the small number of the solvent molecules present in such a small volume. Consequently, fluorescence bursts of single molecules that flow into the probe region can be identify above the background [1, 2].

On the other hand, because of its small focal volume, the LIF based detection has a potential problem with low detection efficiency, which is defined as the fraction of molecules in the analyzed sample that is actually detected. The efficiencies of the on-column detection using conventional capillary columns are often less than 1%. The micro-fluidic chip that we are going to build in the class enhances the detection efficiency by two methods: hydrodynamic stream focusing and electrokinetic focusing. As illustrated in Figure 2, the fluorescent dye labels the flow from the inlet channel (red stream) while nonfluorescent buffer flows from the side channels. The side flow squeezes, or "hydrodynamically focuses," the inlet flow into a thin stream that exits the intersection sheathed in buffer fluid [3].

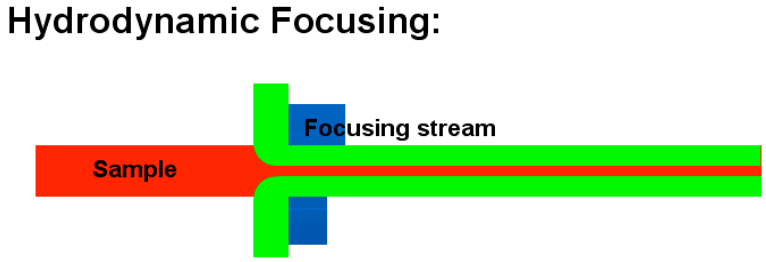


Figure 2. Hydrodynamic focusing by two buffer streams [3]

The focusing width can be controlled by varying the relative pressures driving the side and inlet flows. Many aspects of hydrodynamic focusing can be described by a simple circuit model. The fluid flow can be mapped to a network of resistors.

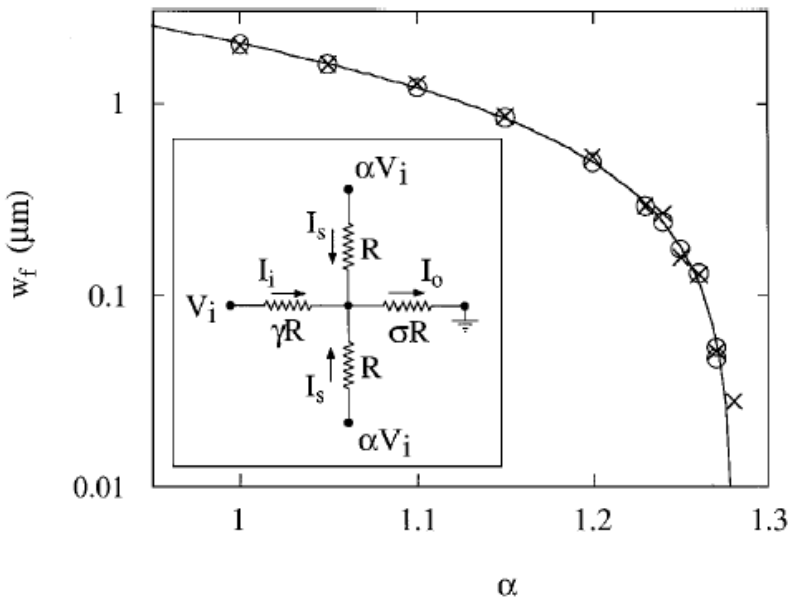


Figure 3. The width of the focused sample stream, w_f, plotted on a logarithm scale against α. α=Ps/Pi, Ps=side (buffer) pressure, Pi=inlet (sample) pressure [3].

Electrokinetics is the study of the motion of bulk fluids or selected particles embedded in the fluids when they are subjected to electrical field kinetics provides effective manipulation techniques in the micro and nano domains, which matches the length scale of biological objects [4 - 7]. Electrophorersis (EP) describes the movement of charged particles in a liquid medium under an external electrical field. When a particle with charge q is under a steady electrical field E, the particle experiences an electrostatic force qE. The electrical force is balanced by a friction force, which can be estimated by Stroke's law, $6\pi\mu Rv$ for a spherical object. The steady state velocity v of the charged particle can be estimated as:

$$v = \frac{q}{6\pi\mu R} E$$

where R is the effective hydrodynamic radius of the particle and μ is the medium viscosity. Thus, negatively charged DNA molecules can migrate under the influence of an external applied electrical field.

When a polarizable particle is subjected to an electrical field, a dipole is induced in the particle. If the electrical field is diverging, the particle

experiences a force that can move it toward high or low electrical field region, depending on the particle polarizability compared with the suspending medium. The process is termed dielectrophoresis (DEP). If the polarizability of the particle is higher than the medium, the force is toward the high field strength region (positive EDP). In the other case, the force is toward the lower field region (negative DEP). The time average dielectrophoretic force is given by:

$$F_{DEP} = 2\pi R^3 \varepsilon_m Re\{K(\omega)\} \nabla E_{rms}^2$$

Where R is the partivle redius, Erms is the root-mean-square electrical field, r is the particle radius, ω is the angular field frequency, Re{k(ω)} represents the real part of K(ω), and K(ω) is the Clausius-Mossotti factor. The Clausius-Mossotti factor is a measure of the effective polarizability of the particle in the medium and is given by:

$$K(\omega) = \frac{\varepsilon^*_p - \varepsilon^*_m}{\varepsilon^*_p + 2\,\varepsilon^*_m}$$

where $\varepsilon^*_{p,m}$ are the complex permittivities of the particle and medium, respectively. For homogeneous particle and medium, the complex dielectric constant is given by:

$$\varepsilon^*_{p,m} = \varepsilon_{p,m} + \frac{\sigma_{p,m}}{j\omega}$$

Electrokinetic focusing:

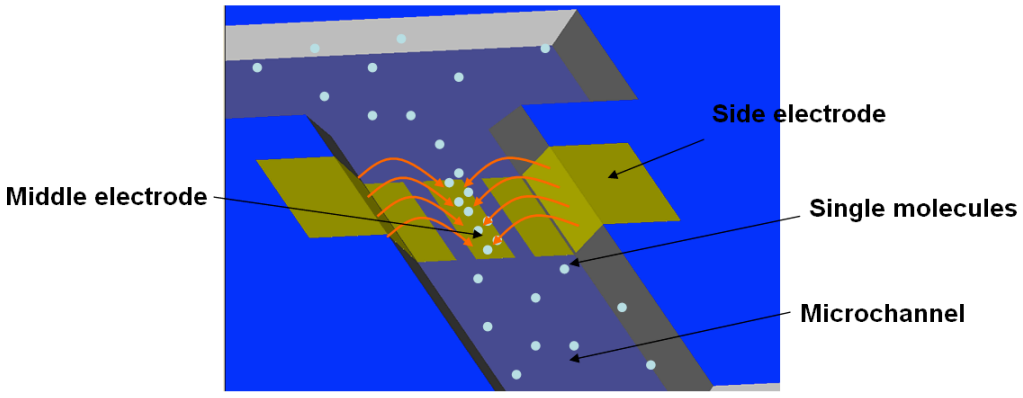


Figure 4. Electrokinetic focusing achieved by 3-D electrodes

FLOW CYTOMETRY

Cytometry refers to the measurement of the physical and chemical characteristics of cells [8]. By extension, flow cytometry refers to the technique where such measurements are made as the cells pass in a fluid stream through a measuring point surrounded by an array of detectors. Although it makes measurements on one cell at a time, it can process thousands of cells in a few seconds. Since different cell types can be distinguished by quantitating structural features, flow cytometry can be used to count cells of different types in a mixture.

Flow cytometers have been commercially available since the early 1970's, and their use has been increasing since then [9, 10, 11]. The most numerous flow cytometers are those used for complete blood cell counts in clinical laboratories -- these do not employ fluorescence. More versatile research instruments employ fluorescence, hence may be distinguished as flow cytofluorometers. Flow cytometric data can be seen in any issue of a scientific journal concerning cell biology.

The cells may be alive or fixed at the time of measurement, but must be in monodisperse (single cell) suspension. They pass through a laser beam by continuous flow of a fine stream of the suspension. Each cell scatters some of the laser light, and also emits fluorescent light excited by the laser. The cytometer typically measures several parameters simultaneously for each cell:

- low angle forward scatter intensity, approximately proportional to cell diameter
- orthogonal (90 degree) scatter intensity, approximately proportional to the quantity of granular structures within the cell
- fluorescence intensities at several wavelengths

Light scatter alone is often quite useful. It is commonly used to exclude dead cells, cell aggregates, and cell debris from the fluorescence data. It is sufficient to distinguish lymphocytes from monocytes from granulocytes in blood leukocyte samples.

Fluorescence intensities are typically measured at several different wavelengths simultaneously for each cell. Fluorescent probes are used to report the quantities of specific components of the cells. Fluorescent antibodies are often used to report the densities of specific surface receptors,

and thus to distinguish subpopulations of differentiated cell types, including cells expressing a transgene. By making them fluorescent, the binding of viruses or hormones to surface receptors can be measured. Intracellular components can also be reported by fluorescent probes, including total DNA/cell (allowing cell cycle analysis), newly synthesized DNA, specific nucleotide sequences in DNA or mRNA, filamentous actin, and any structure for which an antibody is available. Flow cytometry can also monitor rapid changes in intracellular free calcium, membrane potential, pH, or free fatty acids.

Flow cytometers involve sophisticated fluidics, laser optics, electronic detectors, analog to digital converters, and computers. The optics deliver laser light focused to a beam of a few cell diameters across. The fluidics hydrodynamically focus the cell stream to within a small fraction of a cell diameter and break the stream into uniform-sized droplets to separate individual cells (Figure 1).

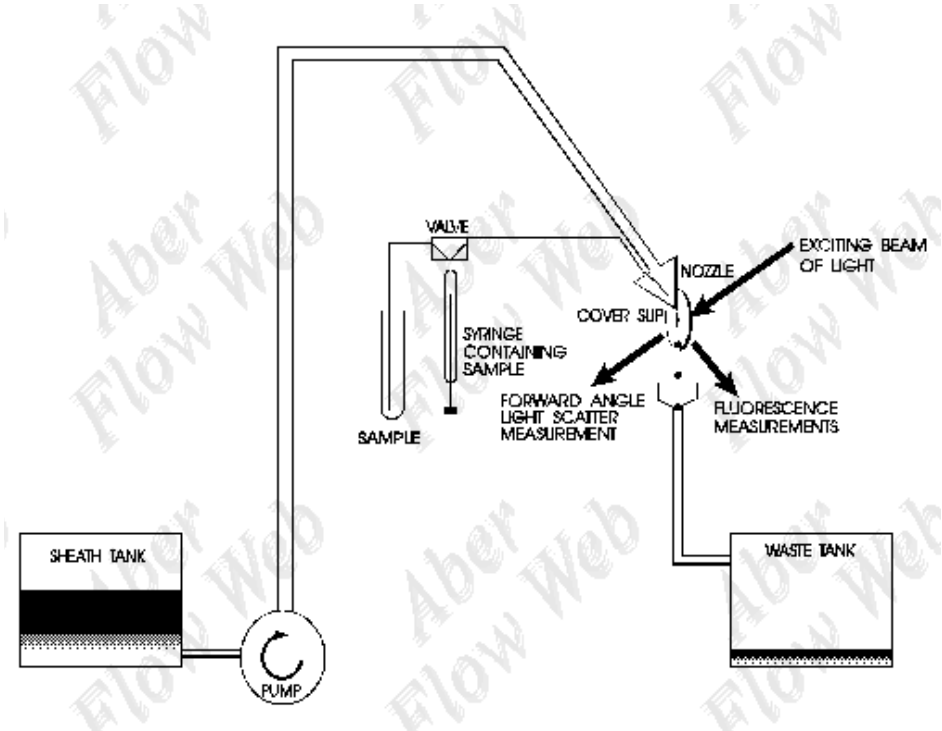


Figure 5. Commercially available flow cytometer [8]

DESIGN AND FABRICATION PROCESS

In the class, we will only focus on the fabrication of micro-fluidic chip and perform the hydrodynamic and electrokinetic focusing of micro particles at the end.

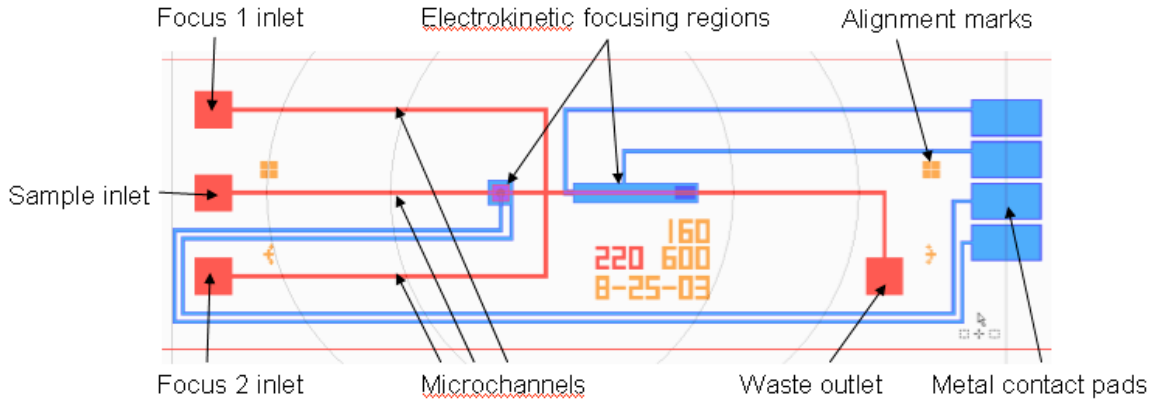


Figure 6. Layout of micro-fluidic chip

The fabrication process begins with a 3” silicon wafer of about 460μm thickness, n-type (100) orientation. Totally three masks are used in overall process.

1. Oxidation of silicon substrate (Lab done by staff)

- Grow 0.5~1μm oxide on silicon surface

2. Patterning the oxide with mask #1 (Lab #2_FC)

- 2μm PR 1813 used as mask
- Buffered HF (BOE) used for wet etching
- Wafer backside also protected by PR 1813 (manually applied)

3. Substrate bulk etching (Lab #3_FC)

- Concentrated KOH used for wet etching
- Temperature control required
- Channel width: 120 ~ 150μm, depth: ~ 20μm

4. Second oxidation (Lab #4_FC)

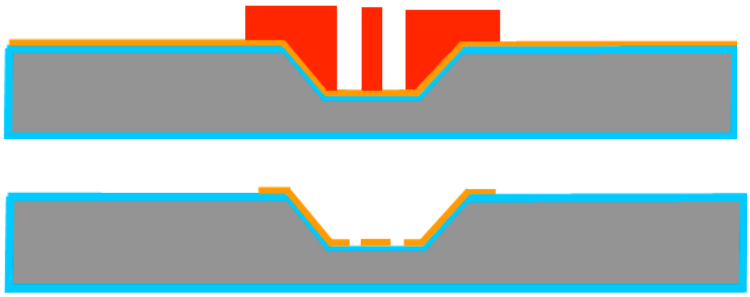
- Grow 0.5μm oxide for electrode isolation purpose

5. Metallization by thermal evaporation (Lab #5_FC)

- 2500Å aluminum film deposited as electrodes
- 10 ~ 20 μm PR SJR5740 photoresist deposition on fresh aluminum

6. Patterning metal by aluminum etchant (Lab #6_FC)

- Photolithography using electrodes mask
- PAN etchant used to remove unwanted aluminum
- Electrodes spacing: 50µm



7. Removal of oxide on after patterning with mask #3 (Lab #7_FC)

- 10µm PR SJR5740 used as mask (Al attacked by BOE)
- Buffered HF used as etchant

8. Anodic bonding with pre-drilled glass plate (Lab #8_FC)

- Pyrex 7740 glass used to seal the microchannels

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