

# Function and Fabrication of Micro-fluidic Chip for Flow Cytometry

**Abstract**— This paper presents the function and fabrication of a microfluidic chip designed for flow cytometry applications, focusing on enhancing the efficiency of laser-induced fluorescence (LIF) detection or any other cell or particle detection system. The project involved a stepwise microfabrication process, including photolithography, anisotropic silicon etching, oxidation, metallization, and anodic bonding—the fabricated device aimed to improve particle detection sensitivity by employing hydrodynamic and electrokinetic focusing techniques. Testing outcomes and potential fabrication improvements are also discussed.

## I. INTRODUCTION

Microfluidics, which manipulates fluids in tiny amounts within microchannel networks, has revolutionized applications in medicine, biology, and healthcare due to its simplicity, low cost, and reduced sample consumption, making it valuable across scientific fields [1]. Microfluidic devices have attracted significant attention for applications such as biological sensing, medical diagnostics, drug delivery, forensic analysis, and molecular detection, where high sensitivity in detecting and identifying molecules and cells is critical. Flow cytometry, a powerful single-cell analysis technology, identifies cell subpopulations and investigates cellular functions by simultaneously analyzing multiple proteins and gene expressions. This technique works by scanning individual cells with a laser beam and collecting the resulting fluorescence and scattered light, ensuring high throughput and enabling the simultaneous measurement of multiple cellular parameters. As a result, flow cytometry provides statistically significant data and insights into single-cell heterogeneity [2].

Microfluidic cytometry devices achieve single-cell analysis by focusing cells or particles into a narrow stream using microfluidic channels. Techniques such as hydrodynamic focusing—where buffer flows compress the sample stream—can precisely control the focusing width by adjusting the sample-to-buffer inlet pressure ratio, as demonstrated by Brian Kirby et al. [3] and James B. Knight et al. [4]. Additionally, electrokinetic focusing enhances particle sorting and detection efficiency by utilizing electric fields. Electrokinetics studies the motion of bulk fluids or charged particles within fluids under electric field dynamics, with electrophoresis (EP) describing the movement of charged particles in a liquid medium under an external electric field [3]. Biological materials, such as red blood cells (RBCs), DNA, and proteins, inherently carry electrical charges; for instance, red blood cells possess a negative charge density of approximately 10 negative ionic groups per cell [5].

Recent advancements in microfabrication techniques, including photolithography, anisotropic etching, and metallization, have enabled the construction of intricate microfluidic networks with micrometer-scale precision [6]. These fabrication methods are essential for achieving effective hydrodynamic and electrokinetic focusing, facilitating high-performance microfluidic flow cytometry. This paper outlines the fabrication process of a microfluidic

chip for flow cytometry, emphasizing the rationale and methodology behind each step.

## II. EXPERIMENTAL

### A. Step-wise Fabrication of the Microfluidic Device

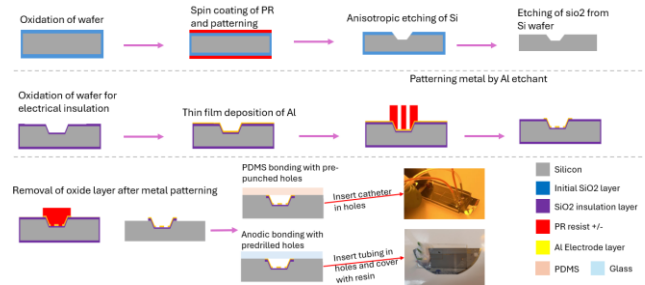


Figure 1: Step-wise Fabrication process.

#### Step 1: Wafer Preparation and Oxidation of Si substrate

Fabrication of the device uses two silicon wafers (wafer A and wafer B) of size 3 inches in diameter and 460  $\mu\text{m}$  thickness (n-type,  $\langle 100 \rangle$  orientation). A layer of silicon dioxide was thermally grown on the wafer surface (by the lab manager before the first lab session). The thickness of the wafer was measured and noted. The oxide thickness of wafer A and wafer B are 3218.6 Å and 7555.3 Å, respectively.

Oxidation can be done using wet or dry oxidation. In this case, the wet oxidation process is used (at 1100°C temperature) as it is faster. The oxide layer thickness is not required to be controlled, so an oxide layer thickness greater than 1000 Å was considered. The oxidation time is 18 min 29 sec for wafer A and 1 hour 20 min 34 sec for wafer B.

#### Step 2: Patterning the Oxide with mask #1

Photolithography was performed to pattern the oxide layer. This patterned oxide layer is used as a mask for Anisotropic Silicon Etching (KOH etching).

The wafers were dehydrated on a hotplate at 150°C for 5 minutes. Positive photoresist (PR S1813) was spin-coated at 2500 RPM on the backside for 60 seconds, and soft baking was performed at 120°C for 5 minutes. Repeat spin coat PR at 3500 RPM on the front side for 60 seconds. Soft baking was performed at 95°C for 90 Seconds.

PR was exposed on the front side on a photomask aligner to UV light for 15 seconds using a darkfield mask and then developed in CD26 developer for 60 seconds. After developing, the wafer was rinsed in DI water and under tap for 30 seconds. Then, it was dried with N<sub>2</sub>. The wafer was hard baked for 2 minutes at 120°C and let it cool for 5 minutes at room temperature in laminar hood.

The developed pattern was transferred to the oxide layer using buffered oxide etchant (BOE) with constant stirring. The time to etch in BOE = thickness of SiO<sub>2</sub>/the Etching rate of BOE (900Å/min). Calculated etch times for wafers A and B are 3 minutes 35 seconds and 8 minutes 23 seconds. The actual etch times are 4 minutes, 30 seconds, and 10 minutes, respectively.

Once the etching is completed, the wafer is cleaned with DI water, dried using N<sub>2</sub>, and inspected under a microscope for defects. PR is removed by immersing it in acetone, followed by cleaning with IPA and drying with N<sub>2</sub>.

#### Considerations:

- The wafer is baked before applying PR to remove moisture and ensure proper adhesion of PR to the Si wafer.
- Soft baking of PR allows it to harden for the exposure step.
- Hard baking of PR allows it not to dissolve during BOE etching.
- Extra protective gear must be worn while handling BOE.
- PR S1813 is a positive photoresist; it gets soluble in the developer when exposed to UV light. In this step, a dark mask instead of a bright field is because we want to remove material in the flow channel pattern. Otherwise, we can use negative PR with a bright field mask.

### Step 3: Anisotropic Silicon Etching (KOH etching)

20 Micron channels were to be etched into the silicon substrate using a 30% KOH solution at 80°C. As depicted in the work of Seidel et al. [7], the etching rate of Si using 30% KOH solution at 80°C is 1.2µm/ min. Before etching in KOH, the wafers are cleaned in the 30:1 HF cleaning solution, then cleaned in DI water and dried with N<sub>2</sub>.

Etching time in KOH is 16 min 40 sec for a 20 Micron channel depth, but the actual value differs. The actual etching rate is calculated by etching the wafer for 10 min. The etching depth is measured as 10.7 µm for wafer A and 11.16 µm for wafer B. These variations happen because of temperature fluctuation and human disturbance while loading and unloading samples in the solution.

Once the etching is completed, the wafer is cleaned with DI water, dried using N<sub>2</sub>, and inspected under a microscope to measure the depth and width of the channel and a profilometer to measure the thickness of the oxide layer for the next steps. Photo records were taken to understand channel defects, surface roughness, etc.

#### Considerations:

- KOH and HF are corrosive. When handling them, Extra protective gear must be worn.
- The etching rate of Si by KOH depends on temperature, fluid agitation, concentration, impurities, and the addition of IPA, which leads to a different etching rate from the calculated one. It is good practice to measure the actual etching rate by stopping the etching in the middle and measuring the depth of the etching.

- IPA is added to KOH to reduce the contact angle between the etching medium and the Si wafer, acting as a wetting agent. Adding IPA to KOH etch improves the surface smoothness of the etched material (Si) by reducing the formation of hydrogen bubbles during etching. This promotes a more uniform etch rate across the surface

### Step 4: Second Oxidation

A second oxidation step is required to achieve a uniform oxide layer thickness for electrical insulation of metal electrodes. After anisotropic silicon etching, the wafer was oxidized again to grow a 0.1 µm oxide layer, providing electrical isolation for metallization.

Before growing the 0.1 µm oxide layer, the previous oxide layer was removed by etching in BOE solution for 3 minutes and 18 seconds for wafer A and 8 minutes and 7 seconds for wafer B. The etching time may need to be extended until all the oxide is removed and the silicon wafer is exposed.

Wet oxidation was performed at 1100 C 1 atm pressure for 3 min 13 seconds to make an oxide layer of 1200Å. The thickness of the oxide layer is measured by color code comparison or using a thin-film measurement system.

#### Considerations:

- Si wafers are fragile, so sudden exposure to high temperatures during oxidation may lead to thermal stress and shattering of wafers. While loading and unloading wafers for thermal oxidation, the wafer is exposed gradually by moving the wafer boat inside the furnace.
- Avoid contamination by touching the wafers, quartz boat, and glass rod to push the boat [8].

### Step 5: Metallization and Patterning of metal with mask #2

Aluminum metal deposit is done using the E-beam evaporation process, in which wafers are placed in an evaporator, and the aluminum substrate is kept in the tungsten boat (0.4 grams of Al is consumed). Aluminum was melted and deposited uniformly onto the wafers at a pressure of 10<sup>-4</sup> torr and a temperature of 1250 C. The layer thickness of the metal after deposition was measured as 463Å.

Post-deposition photoresist (SPR220-7) is spun onto the wafers at 2000 RPM for 60 seconds, baked at 105°C for 7 minutes, and stored in the dark.

For metal patterning, wafers are aligned with a mask in a photo mask aligner and exposed for 100 seconds. The photoresist is developed using CD26 for 15 minutes, rinsed with DI water, dried, and inspected under a microscope. After a 110°C hard bake for 2 minutes, aluminum is etched using PAN (Phosphoric Acid-Nitric Acid) etch (10 seconds), with uniform swirling until patterns emerge, followed by DI water rinsing and drying. The photoresist is then removed with acetone (2 minutes) and isopropanol (IPA 2 minutes). Finally, blow dry using N<sub>2</sub>.

#### Considerations:

- PAN Etch is corrosive. When handling it, Extra protective gear must be worn. This includes long, thick gloves, a rubber apron, and a face

protection shield. All the chemical handling steps except cleaning with DI are to be done in the chemical hood.

#### Step 6: Oxide Patterning with Mask #3 and Cleaning

A third photolithography step selectively removed the oxide layer from all the wafer surfaces except the deposited metal area. This step is done to proceed with the anodic bonding of the Si wafer with glass (Borosilicate or Pyrex glass).

Oxide patterning begins with wafer preparation, where wafers are dehydrated on a hot plate at 120°C for 5 minutes, spin-coated with SPR220-7 positive photoresist at 2000 RPM for 60 seconds, and soft-baked at 105°C for 7 minutes.

During lithography, the wafer is aligned with Mask #3 using alignment keys and exposed to UV light for 100 seconds. The photoresist is then developed in CD26 developer for 15 minutes (a few more minutes based on residuals present on the wafer), rinsed in deionized (DI) water, and inspected under a microscope. This is followed by a hard bake at 105°C for 5 minutes.

Oxide layer etching is done using BOE solution; the etching time is 2 minutes (extra if needed). After etching, rinse in DI water, run under a tap for 30 seconds, and blow dry with N<sub>2</sub>.

Remove PR by immersing the wafer in acetone for 2 minutes, isopropanol for 2 minutes, rinsing with DI water, and drying. The wafer is then stored for subsequent use.

#### Considerations:

- BOE is corrosive; Extra protective gear must be worn when handling it.
- The oxide layer needs to be removed from all wafer surfaces except under metal to promote the anodic bonding of Si with glass.

#### Step 7: Anodic bonding, PDMS bonding, and Packaging

Predrilled borofloat glass with holes at the inlet and outlet with a drill machine. PDMS sheet to be punched with holes at the inlet and outlet of the microfluidic flow channel.

Anodic bonding involves bonding a silicon substrate to predrilled borofloat glass at a temperature of 400°C (a high temperature to break bonds) under a 1000–2000 V voltage (a high voltage to make the ions migrate and create electrostatic force at the interface) for around 10 - 90 minutes (based on the surface evenness and contaminations). This process encapsulates microfluidic channels to allow pressure application during testing. Precise alignment of the glass and silicon is essential, and safety precautions are critical due to the high voltage and temperature involved.

PDMS bonding uses polydimethylsiloxane, prepared by mixing 45 g of monomers with 4.5 g of curing agent (10:1 ratio). It is cut to fit the chip after curing the PDMS at 80°C for 2 hours. The PDMS and chip are exposed to plasma for 1 minute to activate their bonding surfaces, which are then

quickly joined together. This method leverages plasma activation to create a strong bond between the PDMS and the chip.

While anodic bonding is suitable for robust, permanent packaging with borofloat glass, PDMS bonding is simpler, more flexible, and better suited for prototyping or temporary setups.

For an anodic bonded chip, insert tubes into the inlet and outlet holes, add resin to seal the fluid flow channels, and add syringe lure adapters on the other end of the tubes.

For a PDMS-bonded device, insert a metal needle into the catheter device, which can be connected to a syringe.

#### *B. Chemicals used and preparation of solutions*

- PR S1813 [9]
- SPR220-7 [10]
- CD-26 Developer [11]
- Acetone [12]
- IPA - Isopropyl alcohol or Isopropanol [13]
- Deionized water
- N<sub>2</sub>
- O<sub>2</sub>
- Aluminum
- PDMS + Curing agent [14]
- Buffered Etch Oxide (BOE)

To prepare a buffered oxide etch (BOE) solution, weigh out 296 g of ammonium fluoride (NH<sub>4</sub>F) and add it to 425 mL of deionized (DI) water in a 1000 mL plastic beaker while stirring. Slowly add 106 mL of hydrofluoric acid (HF) to the mixture, ensuring careful handling to prevent splashing or heat generation. Transfer the prepared BOE into a Teflon petri dish for use.

Caution: Wear appropriate PPE (gloves, goggles, face shield, and lab coat) throughout the process. HF and NH<sub>4</sub>F are hazardous chemicals considered highly corrosive; they seep into the skin and cause bone damage.

- KOH Etch

Prepare a 30% KOH etching solution by weighing 250 grams of KOH pellets and adding them to a beaker containing 500 ml of deionized (DI) water. Gently agitate the mixture on a warm plate until the KOH pellets are dissolved, then add approximately 2.5 ml of isopropyl alcohol to the solution. If not used immediately, store the solution in a plastic bottle labeled with your name, date, and "30% KOH."

- PAN Etch

To prepare a Phosphoric Acid-Nitric Acid (PAN) etch solution, mix 8 parts phosphoric acid (85% concentration) with 1-part nitric acid (70% concentration) in a chemical hood, stirring carefully to ensure uniform mixing. Then, add 1 part of deionized (DI) water to the mixture while stirring. The solution is typically heated to 120–150°C to enhance its etching performance, particularly for silicon nitride, with minimal impact on silicon dioxide. Always add nitric acid to phosphoric acid (never reverse) to avoid exothermic

reactions and ensure safety. Use proper PPE, including acid-resistant gloves, goggles, a face shield, and a lab coat, and work in a well-ventilated area. Dispose of the etchant safely following hazardous waste protocols [15].

### III. RESULTS & DISCUSSION

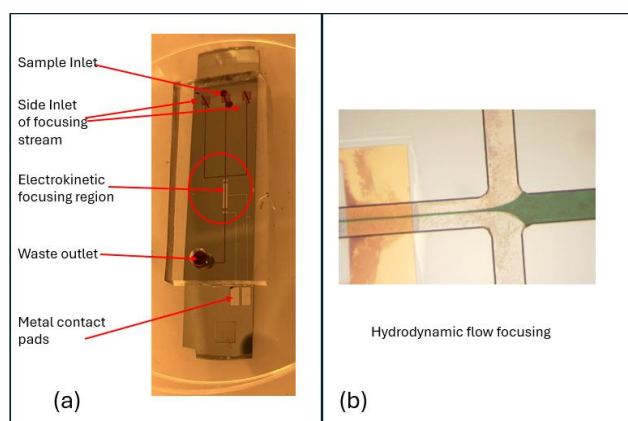


Figure 2 (a) Features of the final device, (b) Testing of the device and flow focusing using hydrodynamic principle.

Two packaging methods, anodic bonding, and PDMS bonding, were tested for their ability to seal and maintain fluidic flow under pressure using manually controlled syringes.

Anodic bonding, which utilized pre-drilled borofloat glass sealed with adhesive, exhibited robust performance with no leakage under constant pressure, demonstrating superior reliability and functionality.

In contrast, PDMS bonding, where bonding between PDMS and glass plate showed insufficient bonding strength and leaked midway through testing, highlighting its limited durability. The fabricated microfluidic device successfully demonstrated hydrodynamic focusing, with buffer streams compressing the sample stream into a narrow path.

However, some challenges were observed, including surface roughness from inconsistent etching and minor misalignments during mask exposures. Future improvements include upgrading alignment tools, refining etching parameters, and enhancing electrode functionality by incorporating aluminum electrodes for advanced flow focusing and particle manipulation [16].

Additionally, adopting innovative focusing techniques, such as inertial focusing, Dean flow, and magnetic or acoustic cell focusing [17], alongside design modifications like altered dimensions and sorting features, could enhance device performance.

Overall, anodic bonding was the preferable sealing method, offering a reliable foundation for advancing microfluidic applications.

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