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A Microfluidic Device for Capturing Circulating Tumor Cells

THESIS

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in Biomedical Engineering

by

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ABSTRACT OF THE THESIS

A Microfluidic Device for Capturing Circulating Tumor Cells

By

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Circulating tumor cells (CTCs) are cells that shed into the vasculature from a primary tumor and circulate in the bloodstream. CTCs can be used to elucidate the molecular characterization of the tumor cells and to gauge the efficiency of therapeutic treatment in metastatic carcinoma patients. They can also be used to determine the primary site of the tumor in areas where the tumor is undetectable with traditional oncological imaging. The detection of CTCs has a substantial value for prognostic and therapeutic implications, but they are not easily detected because of their low cell count. Because microfluidic devices are useful for cell detection and diagnosis, can be easily obtained, and are less invasive than tissue biopsies, we have developed a microfluidic platform to capture CTCs using multiple capture targets to achieve a higher cell capture. We can selectively isolate the cancer cells using specific antibodies to the antigen capture target on the surface of malignant cells. The capture efficiency was evaluated by the flow rate, cell count, and antibody immobilization. Cancer cell lines that were known to have high expression for targeted ligands, specifically HER2, EGFR, EpCAM, and MUC-1, were tested with antibodies specific to these ligands. We
obtained capture efficiency with these different capture targets on a single channel. This allowed us to develop a device with four parallel capture channels to run in series with the anticipation of achieving higher cell capture.
CHAPTER 1

Introduction

1.1 Motivation

Cancer is a disease of abnormal cell proliferation due to loss of cell cycle controls. This disrupts the organization of tissues to form lumps or masses known as tumors. Tumors can grow in and interfere with multiple body systems, including the digestive, nervous, and circulatory systems, as they release hormones that can alter body function [1]. The American Cancer Society projects that over 1.5 million people will be diagnosed with cancer this year and a third will die of cancer in United States. The majority of these cancers are metastatic, spreading from one part of the body to another. Patients with these types of cancers including—breast, kidney, and lung cancer—have tumor cells moving throughout their circulatory system known as circulatory tumor cells (CTCs) [2].

CTCs are invasive, motile, and adaptable in foreign environments. In cancer development, tumor cells can grow and lose their ability to adhere to one another within the tumor. These mobile CTCs can be transported to other parts of the body through the bloodstream. When attached at a different site, CTCs are capable of growing new blood vessels to proliferate and survive. These abilities allow CTCs to leave their primary tumor and start a secondary tumor elsewhere in the patient’s body [3]. Consequently, the cancer in the patient evolves into a much more dangerous circulating carcinoma that can exponentially worsen over time. This is often the cause of metastasis in patients, which is the cause of the majority of cancer-related deaths. Therefore, CTCs are critical in cancer
research as the majority of cancer patients die from the secondary, rather than primary tumor.

The isolation of CTCs is challenging due to a low cell count of approximately one CTC per \(10^9\) hematologic cells [4]. Microfluidic technology has attracted research interest for capturing CTCs because of its higher capture yield for these rare cells, ability to preserve cell viability, and allowance for detailed molecular and functional characterization [5]. Detection of CTC levels allows healthcare professionals to diagnose, detect, and monitor the progression of the patient’s cancer and adjust their treatment accordingly without invasive biopsy.

1.2 Background

Cancer Biology

All living organisms are composed of cells, the “building blocks of life” that grow, divide, and die in a controlled mechanism for the organism to function properly. Uncontrolled growth, the hallmark of cancer, results from biochemical alterations in normal cells that inhibit proper cell regulation [6].

Cancer cells continuously proliferate since they are not differentiated. Furthermore, they elude apoptosis or programmed cell death, which the body uses to remove unwanted cells. These abnormal cells grow to form a mass of tissue, known as a tumor, which can either be benign or malignant. Benign tumors are noncancerous masses that grow slowly and do not spread or invade other parts of the body, whereas malignant tumors grow rapidly and invade nearby tissues and organs. A primary tumor develops at the original site of tumor formation. Malignant tumor cells may escape the primary mass, travel through the
circulatory system, then invade the vascular basement membrane of the new site in the process of extravasation. Ultimately, these cells will colonize and proliferate to form secondary tumors or metastases. These secondary tumors may sustain themselves, then expand, invade and damage nearby tissues [7]. Invasion and metastasis (Figure 1) are very closely correlated as they are crucial to the fatality of most cancers. The presence of metastatic disease is the major cause of cancer-related death in patients with solid tumors [8].

Metastasis has been associated with CTCs, tumor cells circulating in peripheral blood. CTCs are malignant cells that originate from primary tumors and migrate to distant sites through the hematogenous and lymphatic systems [9]. These cells extravasate at the site of interest, colonize and develop a new malignant tumor. Studies show that changes in CTC levels in the blood correlate with tumor progression in patients. The advantage of capturing and counting CTCs is that it requires only a small sample that can be easily obtained as opposed to traditional invasive biopsies [10].

![Figure 1 The Invasion-Metastasis Cascade](image-url)
Microfluidic platforms provide a great clinical assay that allows for more effective and efficient cell separation despite the small size of individual human cells. This has several important advantages including reduced sample volumes, faster processing time, high sensitivity and portability [12]. As a result, this will be beneficial for isolating rare cells, which can ultimately be used to reduce the mortality due to metastasis [13].

**Molecular Tumor Markers**

The surface of malignant cells overexpress membrane antigens unique to the tumor. Antibodies can be used to bind to those specific antigens. This allows for the selective isolation of cells from a cancer line bearing the antigen-antibody complex [14].

EpCAM is a transmembrane glycoprotein on epithelial cells that plays a significant role in tumorigenesis and metastasis of carcinomas. CTCs, although originating from epithelial cells, often have higher levels of EpCAM on the membrane surface compared to normal cells. For example, EpCAM expression have been measured to be greater than one million per cell in HCT116 colon cancer cells, H1650 lung cancer cells, and SK-OV-3 ovarian carcinoma [15]. Subsequently, EpCAM can be used as diagnostic marker for various cancers since they are overexpressed in most human carcinomas.

Other membrane proteins that are also overexpressed by cancer cells are human growth factor 2 (HER2), mucin-1 (MUC-1), and epidermal growth factor receptor (EGFR). HER2 promotes the growth of cancer cells and has high expression level in many types of cancers, including breast cancer [16]. MUC-1 is highly overexpressed and glycosylated in a variety of carcinomas [17]. EGFR exists on the membrane surface and its activity is increased in cancerous cells [18]. CTCs that have high expression levels for these
membrane proteins can be used to detect, characterize, and monitor non-hematologic cancers.

**Cancer Diagnostic Technologies**

There are several diagnostic technologies used to assess cancer such as conventional radiologic imaging techniques, molecular detection, and immunomagnetic-based isolation. However, all these methods are expensive, require a large sample and a long sample preparation time. In contrast, miniaturized devices that can isolate CTCs will be more ideal since they have a higher throughput, are cost efficient, and require a comparatively smaller sample. Also, an integration of multiple systems can be achieved on the same device, making it more like a point of care operation [5].

Microfluidic devices have been commonly used in the last decade to separate, sort, and isolate CTCs. A number of methods have been described to isolate CTCs based on physical characteristics and capture via specific biomarkers, with the latter attaining clinical success. The CTC chip was reported to be able to selectively isolate CTCs from peripheral whole blood samples. The CTCs were originally captured onto the microposts that are conjugated with EpCAM. However, the herringbone chip, a more recent and redesigned version of the CTC chip, was reported to have improved capture efficiency. In the herringbone device, the channel surfaces are functionalized with EpCAM antibody using avidin-biotin chemistry. Subsequently, biotinylated antibodies can capture EpCAM expressing cells as they pass through the channels [19].
CHAPTER 2

Design and Fabrication of CTC Capture Device

2.1 Overview

Our design for a herringbone device was based on a microvortex-generating herringbone device developed by Mehmet Toner’s research lab in the Harvard Medical School. They made a microfluidic device with which they increased capture efficiency with the use of optimal dimensions and flow rate [19]. Our lab used their device design for use with multiple capture targets.

This design allows the cancer cells to travel through the device at a flow rate that results in continuous interaction between the cells and the channel walls. The surfaces of the channel walls were conjugated with antibodies that have high expression to the capture cells, therefore a higher capture rate could be achieved by maximizing interactions of cells with the functionalized channel walls. The purpose of the herringbone structures micropatterned onto the top surface of the channels was to promote mixing with vortices generated from non-uniform flow paths at the optimal flow rate.

2.2 Evolution of the Device

Our design has gone through several iterations, investigating a variety of methods and comparing their advantages and disadvantages. First a CD microfluidic device with the use of a Zeiss spin stand was implemented. This initial CD device originated from our previous BME180 project. The original goal was to develop a more automated system. However, it was not ideal for the low flow rate needed to obtain a high capture efficiency
implied that the rotation rate of the spin stand must be set at a level far too low for stable operation. The final design that allowed for a multi-capture targets of CTCs was a linear design with the use of a syringe pump. A photo of the final design of the prototype can be seen in the schematic below in Figure 2. We first demonstrated an acceptable level of capture efficiency by using separate channels, each functionalized with different antibodies for its corresponding high expression of cell line. Once we were able to achieve high capture efficiency, we would then integrate the channels into a single-pass CTC capture platform that would isolate multiple targets. The current work paves the way for this future device.

Figure 2 Herringbone Channel Device
Symmetrical channel with an inlet and outlet. The herringbone structures are facing down from the top surface and adhered onto the glass cover slip.
Stage 1

The first iteration of the design was a CD microfluidic device; it demonstrated versatility in integrating transporting, mixing, and processing functions within the platform. This CD microfluidic design had forty channels in order to increase the throughput. These channels are illustrated in Figure 3 below. The design allows the device to be spun such that the centrifugal force causes the fluid to flow through to the outer perimeter of the CD. The herringbone design of the channels causes mixing of the fluids (described further in Fluid Flow), and increases the probability of interaction between the CTC and the inside channel walls. From this SolidWorks model we were able to 3D print a prototype.

Figure 3 Collapsed and Exploded Views of the 3D SolidWorks Generation 1 Model

In the Left image assembly view: CD device with all five compartments sealed together. In the right image exploded view from top to bottom: top cartridge, PDMS fans, capture slip, rubber piece, and bottom cartridge.
During the prototype validation of the stage 1 device, we noticed internal leakage that did not allow us to perform experiments. The fluid was inserted into the inlet hole; however, not all the fluid was able to flow through the channels without leakage. Thus, the fluid did not easily or completely empty out into the outer ring of the device. The leakage could have been due to insufficient rigidity of the device since there were so many channels incorporated into the design, and the channel seal relied entirely on clamping the top and bottom pieces.

**Stage 2**

Since the first iteration of the CD design experienced leakage during prototype validation, we decided to resolve the leakage by simplifying the device. The second iteration of the CD microfluidic device had only 8 channels instead of 40 allowing a more structurally rigid design. These channels are illustrated in Figure 5. All the components of the new iteration were more secure, which allowed the device to mechanically seal better. The sidewall thickness were increased so that the channels could be held more securely to prevent the leakage experienced during the first iteration. However, we still had some
leakage during prototype validation although the amount of leakage was reduced from the initial phase.

A CD device was mainly used for faster processing. A low flow rate could not be achieved with this CD design, which was not ideal to capture rare cells from heterogeneous solution. This resulted in non-optimal capture efficiency. We decided to completely change our design not only because continuing to develop methods to prevent internal leakage would be non-trivial and time consuming, but also because a CD device was not best for antigen-antibody bindings.

**Figure 5 Collapsed and Exploded Views of the 3D SolidWorks Generation 2 Model**

Left image assembly view: CD device with all five compartments sealed together.
Right image exploded view from top to bottom: top cartridge, PDMS fans, capture slip, rubber piece, and bottom cartridge.
Final Design

Our final design combined aspects of the herringbone grooves with a linear design with the use of a syringe pump. It uses a simple linear channel (Figure 7) through which the fluid would flow and interact with the channel geometry. The pump provided a pressure difference across the channel and flowed fluid at a set volumetric rate that was optimized for high turbulence and high capture efficiency for antibodies conjugated on the surfaces.
2.3 Design Parameters and Requirements

Fluid Flow

It was imperative to flow the sample containing CTCs at a flow rate that provides a high capture efficiency. A high flow rate can cause the cells to have minimal interaction
with the surfaces of the microchannel while it flows through the device. This can result in a low capture efficiency of rare cells because the cells are required to have maximal contact with the surface. A lower flow rate allows for a higher number of cell-surface interactions in the antibody-coated device. The optimal low flow rate of 2 µL/min through the microchannel was established by Toner’s lab [19].

There are two main forces for driving the flow of fluids in microchannels in the PDMS devices, which are pressure-driven and electrokinetic. The flow rate in a pressure-driven flow is Q (m³s⁻¹). Q is given by Q=ΔP/ R, where ΔP is the pressure drop across the channel (Pa) and R is the channel resistance (Pa-sm⁻³). The pressure drop can be created either by opening the inlet to atmospheric pressure and applying vacuum at the outlet, or by applying positive pressure at the inlet and opening the outlet to atmospheric pressure. For our experiments, we used a pressure-driven flow by opening the inlet to atmospheric pressure and applying negative pressure at the outlet using a syringe pump. A syringe pump has a driven flow that involves a high fluid flow pressure, thus it is necessary to form a permanent seal between PDMS mold and glass slide [20].

For pressure-driven flow, the other factor for flow rate is the channel resistance R. The microchannel resistance for a rectangular channel with a high or low aspect ratio, (channel width << channel height) or (channel width >> channel height) with constant cross section along the entire length, is given by \( \frac{\mu L}{w h^3} \), where \( \mu \) is the fluid viscosity (Pa.s), L is the length (m), w is the width of the channel (m), and h is the height [1]. High pressure drops are needed to drive fluid flow in microchannels since the fluid resistance is inversely proportional to wh³ [20].
**Laminar Flow**

In microfluidic devices, the flow of fluids in a microchannel is generally laminar. Laminar flow is used for cell biology in PDMS microchannels to deposit proteins or cells. The motion of the particles of fluid in a laminar flow is orderly with all particles following streamlines and displaying minimal molecular diffusion across flow channels. This absence of mixing results in a limited amount of contacts with antibody-coated surface of device, which is crucial for target cell capture. Herringbone design structure was successfully demonstrated by the Toner Lab at MIT with encouraging results in capturing CTCs. This design uses a geometry within the flow path to induce chaotic mixing at low Reynold numbers and causes microvortices. This potentially increases the amount of CTC incidents on the surface of the microchannels and leads to a higher capture rate of rare cells [19].

### 2.4 CTC Microfluidic Device

**Design**

The final dimensions of the herringbone structures were selected based on the optimization studies conducted from the Toner Lab by varying the ratio of height of the grooves to that of the channel, herringbone dimensions, and periodicity. The optimal height of the herringbone was 50 µm with a displacement of 50 µm inside the channel. The overall height of the channel was 50 µm, with a total height of 100 µm in the un-grooved areas [19]. The width and length of the channels were set to take up the entire 1” x 3” glass slide and allowing it to fit two channels. The dimensions for the design are illustrated in Figure 9.
Simulations

We utilized COMSOL, a finite element analysis program, to study the flow characteristics of our design before fabrication. COMSOL’s inert programming allows three-dimensional mold of the channel to transfer from SolidWorks. Flow fluid property and wall conditions like flow inlets and outlets can be set along with initial flow velocity and appropriate meshing. Fluid mechanics equations like Navier-Stokes and continuity are built into COMSOL’s framework. While channel conditions, fluid and material properties can be specified. The simulations use these conditions to calculate conditions like velocities, particle distributions, and flow visualization.
The mold of the channel allows COMSOL to identify the imported model as one solid object of a defined material. Since the channel is symmetrical, the model was cut down in the middle so that only half of the device needed to be simulated (see Figure 10). Once the model was imported and the material selected, the mesh had to be defined. For simple models, a general mesh can be used. These meshes can range from extremely coarse to extremely fine; the finer the mesh, the more precise the results. However, due to the complexity and number of the herringbone arrays, using a general mesh would produce errors. Thus, the model required a user-defined mesh.

The user-defined mesh consisted of several free-triangular meshes and one free-tetrahedral mesh. The herringbone structures were meshed in stages using a normal free-triangular meshes calibrated for fluid dynamics. The three stages meshed faces 1-10,000,
10,001-20,000, and 20,001-24650 respectively. An extra fine free-tetrahedral mesh calibrated for fluid dynamics was applied to the remaining, unmeshed faces of the model. Once the mesh was completed, the laminar flow study was set up.

The standard COMSOL laminar flow fluid property settings and wall conditions were used for the simulation. The inlet was defined with a laminar inflow boundary condition and volumetric flow rate of $3.33 \cdot 10^{-11} \text{m}^3\text{s}^{-1} (2\mu\text{L/min})$. Taking advantage of the structural symmetry, a symmetry boundary condition was included in the center plane of the channel to reduce the simulation time by half. Standard settings were applied to the outlet. Once the laminar flow conditions were set up, a stationary study was developed.

The stationary study computed the results of laminar flow through the model. The

![Figure 11 Velocity Simulation Output](image)

The velocity of each stages through the channel was simulated via COMSOL. Brighter colors (green, yellow, red) represent higher velocity. Dark colors (blue and dark blue) represent little to no velocity.
standard study settings apply an iterative solver, which failed to converge. To avoid these errors, a direct solver was applied instead and the desired results were produced. The results showed the velocity gradient along the device. Selection of colors corresponding to levels of velocity can be visualized as shown in Figure 11. The brighter colors (green, yellow, red) represent higher velocity, while the dark colors (blue and dark blue) represent little to no velocity.

Particle tracing for fluid flow was used to simulate the CTC particle flow through the model. The inlet and outlet settings were linked to the laminar flow settings. The particles were set to stick to the walls of the model to simulate capturing the CTCs. An exception was made to the inlet in order to prevent the particles from being captured in the inlet of the device preventing them from flowing through the model. A drag force was added, as this was the force moving the CTCs through the model. The velocity field for the drag force was linked to the velocity field of the laminar flow. Gravity was also included but it had a small effect on the flow of the particles. Figure 13 shows the particle themselves and where they were captured inside the channel.
The particles flow pattern through the channel from the inlet on the left towards the channel with the herringbone structures was created via COMSOL time-dependent study.
A second, time-dependent study was added to produce the particle tracing results. The result shown in Figure 12 shows the velocity profile of the flow through the channel. The velocity profile of the flow displays the chaotic microvortices generated by the herringbone grooves.

From these simulations, we were able to further understand how the geometric design of the herringbone ridges in our channel causes passive mixing of the fluids through the formation of microvortices that the Toner group demonstrated. The particles exhibit frequent upward and downward motions inside the channel while flowing towards the outlet. The simulation studies also displays how the particles are mostly captured on the bottom surface of the channel while some are also captured on the herringbone grooves facing downwards from the top surface.

**Fabrication**

We decided to use conventional photolithography processes to fabricate our mold since they are commonly used for patterning microfluidic systems. Photolithography is a process of copying designs from a mask into thin light-sensitive polymeric films (photoresist) that can be spread out onto a substrate like silicon wafer. The master mold was made using SU-8 (shown in **Figure 15**), which is a negative photoresist and the channels on the device were fabricated with polydimethylsiloxane (PDMS). This is a very common method for creating microstructures in academia. PDMS is a two-part silicon-based organic polymer that provides a quick, easy, and cheap way of making the fluid channels.
Figure 14 Diagram of PDMS Fabrication Process

Cross section view of the channel 
A) The first spin is for a 50 μm layer of SU-8 2050, 
B) The first exposure is done with Mask 1 to pattern the channel section, 
C) The second spin is for a 50 μm layer of SU-8 2050, 
D) The second exposure is done with Mask 2 to pattern the herringbone structures in the channel, 
E) The SU-8 is developed to release the features of the mold, 
F) PDMS casting from silicon wafer, 
G) PDMS is adhered to a glass slide.
2.5 Material and Methods

Silicon Wafer

We chose to print the photomasks for the mold of the CTC capture device through Output City since they specialize in making photomasks. The mold consisted of two layers of photomasks created on SolidWorks. The file was converted to AutoCAD and sent to Output City for printing. The patterns for the mold were printed on an 8x10 inch at a resolution of 20k to achieve the minimum feature size of the design. The photomasks were then used to make the silicon wafer by photolithography. Photolithography allows for small feature sizes, down to 10 μm, for our devices at a comparatively low cost.

Figure 15 Silicon Wafer of the CTC Mold
Protocol followed for photolithography:

1. Substrate Preparation
   - Rinse silicon wafer with either acetone, methanol, IPA, or DI wafer
   - Dehydration bake for 1 hour at 120 °C
   - HMDS surface treatment on wafers using YES Oven

2. SU-8 application for layer 1 using Laurel spinner using mask 1 to achieve a thickness of 50 μm using SU-8 2050
   - Spin 500 rpm for 10 seconds at 100 rpm/s
   - Spin 2200 rpm for 20 seconds at 300 rpm/s

3. Soft Bake
   - Bake wafer for 3 minutes at 65 °C and then 4 minutes at 95 °C.

4. Exposure
   - Apply 160 mJ/cm² with 12 mW lamp for approximately 13.3 seconds

5. Post Exposure Bake
   - Bake the wafer at 95 °C for 10 minutes

6. SU-8 application for layer 2 using Laurel spinner using mask 2 to achieve a thickness of 50 μm using SU-8 2050
   - Spin 500 rpm for 10 seconds at 100 rpm/s
   - Spin 2200 rpm for 20 seconds at 300 rpm/s

7. Soft Bake
   - Bake each wafer at 95 °C for 10 minutes

8. Alignment
Go to Alignment tool and align mask 2 over the first exposure

9. Exposure

- Apply 160 mJ/cm² with 12 mW lamp for approximately 13.3 seconds

10. Post Exposure Bake

- Bake each wafer at 95 °C for 10 minutes

11. Development

- Develop in SU-8 Developer solution for 25 minutes

12. Rinsing

- Rinse with fresh SU-8 developer solution then with IPA
- Rinse with DI water
- Place in wafer carrier

13. Hard Bake

- Place wafers on a hot plate and slowly ramp up heat from room temperature to 65 °C, 90 °C, 120 °C, 150 °C, and then ramp back down from 150 °C, to 120 °C, 90 °C, 65 °C, and then back to room temperature.
AutoCAD drawings of Mask 1 and Mask 2 designs. Mask 1 polarity matches to pattern the channel. Mask 2 polarity matches to pattern the herringbone structures on the channels. The micropillars were included to provide support and prevent the channel from collapsing.
Silanization of the Wafer

Silanization was necessary since PDMS binds strongly to the thin native oxide layer that is normally present on the surface of silicon wafers, making it difficult to peel away the cured PDMS from the silicon master mold. Thus, the silicon master mold needed to be silanized prior to casting PDMS using trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma-Aldrich). The wafers were placed in a vacuum chamber and the silane evaporates in the chamber for 24 hours. This allowed for a surface reaction to occur where the silane preferentially binds to the oxide layer. This causes a fluorocarbon residue to stick up from
the wafer’s surface and preventing the PDMS from strongly bonding to the silicon wafer [21]. The molds were ready to be cast with PDMS once this process is completed and should be repeated after 3-5 castings.

**Device Making**

The PDMS device was made by mixing the silicon elastomer base (Sylgard 184, Dow Corning) resin with a cross-linker (Sylgard 184, DowCorning) at a ratio of 10:1 by weight. This solution was mixed vigorously to form a prepolymer and then poured over the silicon molds. All the features on the wafer were covered with the mixture to come up to a thickness of about 5 mm. The wafer was placed in a vacuum chamber for at least 30 minutes to remove all air bubbles that were trapped between the surface of the molds and the prepolymer. Then the molds are placed in a 70 °C oven and left for at least 2 hours to cure. Once the cross-linked PDMS castings (formed via hydrosilylation reaction between vinyl groups and hydrosilane groups) were formed, the design was ready to be cut. The designed microstructures were cut on the border marks using a razor blade and peeled off from the silicon mold using tweezers. Holes of diameter 1.5 mm were pierced into the PDMS device using a borer to produce inlets and outlets.

The device consisted of PDMS microstructure and a glass slide that required a permanent bond. We used oxygen plasma bonding to irreversibly bond the two layers since it is the most common method used. The glass slide was first rinsed with either isopropanol or ethanol in order to remove any dust or other particles residing on the surface. The PDMS device was bound to pre-cleaned 1” x 3” glass slides by exposing the binding surfaces of
PDMS channels and glass slides to air plasma for 2 minutes. The plasma treated device was then placed in the oven for a minimum of 1 hour to form the final devices.

**Tubing**

The tubing that was inserted in the inlet and outlet openings has explicit specifications. They were manufactured by Fisher Scientific and made of polyvinyl chloride (PVC). The inner diameter of the tubing is .020 inches with a thickness of .020 inches.

A specific length of tubing is inserted in either the inlet or out of the device opening. The inlet of the device had a tubing length of 29 cm while the outlet was 13 cm. The tubing length was set according to the time required for the fluid to prime the inlet of the tubing and needed time to exit the device without creating pressure inside the device. The volume of the liquid needed to prime the inlet during the experiment is as follows:

\[ V = \pi \times (\text{Inner radius of the tube})^2 \times \text{Length of tube} \]

**Syringe and Needle**

The syringes used for the experiments were latex free (Fisher Scientific). It held a volume of 1 mL and had an outer diameter of 4.78 mm. The needle that was placed onto the syringe was 25 gauge with a length of 5/8” (Fisher Scientific). The sharp edge of the needle was removed with a cutter before it was inserted into the tubing.
CHAPTER 3

*In Vitro Cell Experiment*

### 3.1 Introduction

In order to test the CTC capture device with cancer cells, the microfluidic channels were immobilized with antibodies using avidin-biotin chemistry. The channels of the devices were functionalized with biotinylated antibodies like anti-EGFR, anti-EpCAM, anti-HER2, and anti-MUC-1 protein molecules. The cell lines corresponding to the antibodies were A431 epidermoid carcinoma, H1650 lung carcinoma, and SK-OV-3 ovarian carcinoma respectively. These cells have already been studied to express high antigens complementary to its targeted ligands. The cancer cells were appropriately spiked in PBS+ buffer at a concentration to allow 1,000 cells to be passed through the functionalized channels. A control channel was not functionalized with antibodies and was run in parallel with the experimental channel to confirm no cell capture. The device test concludes the capture efficiency for different capture targets.

### 3.2 Materials and Methods

**Cancer Cell Culture**

We tested four cancer cell lines using the CTC capture device. The cell lines were as follows:
Table 1 Cancer Cell Lines

<table>
<thead>
<tr>
<th>Cancer Cell Line</th>
<th>Cancer Cell Type</th>
<th>Cell Media</th>
<th>Trypsinization Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431</td>
<td>Epidermoid Carcinoma</td>
<td>CDMEM</td>
<td>12-15</td>
</tr>
<tr>
<td>H1650</td>
<td>Lung</td>
<td>RPMI</td>
<td>4-6</td>
</tr>
<tr>
<td>HeLa</td>
<td>Cervical</td>
<td>CDMEM</td>
<td>6</td>
</tr>
<tr>
<td>SK-OV-3</td>
<td>Ovarian</td>
<td>RPMI</td>
<td>6</td>
</tr>
</tbody>
</table>

Materials

14. C-DMEM (DMEM, 10% FBS, 1% P/S, 1% L-Glu) and RPMI-1640,

15. Hank’s Balanced Salt Solution (HBSS)

16. Trypsin-EDTA

Materials from Fisher Scientific

Protocol

1. Warm Trypsin-EDTA and cell media in 37°C water bath for approximately 30 minutes.

2. Observe the desired cell line under 10X objective Olympus microscope to ensure desired confluency has been achieved.

3. In a tissue culture hood, aspirate media from the flask containing the cells.

4. Wash the cells with 5 mL of HBSS and aspirate HBSS from the flask.

5. Cover the entire surface area of the flask containing the cells with 3 mL of trypsin.
6. Place the T75 tissue culture flask in 37 °C, 5 % CO₂ incubator for the time required for the cells to come off the bottom of the flask. The incubation time is dependent on the cell line.

7. After the incubation, observe the cells under the microscope to make sure that the cells are detached and floating on the surface.

8. Place flask in culture hood, add 7 mL of cell media to the flask. Vigorously pipet the entire solution up and down to obtain a homogenous mixture.

9. Transfer the 10 mL solution to a 15 mL conical tube.

10. Spin the conical tubes in the centrifuge at 1.5G for 3 minutes; a cell pellet should form at the bottom.

11. In the culture hood, aspirate the supernatant above the cell pellet. Be careful to not disturb the pellet.

12. In a fresh T75 tissue culture flask, add 14 mL of media.

13. Break up cell pellet by adding 5 mL of cell media to the conical tube. Pipet up and down until a homogenous solution is achieved (try to avoid creating bubbles).

14. Add 1 mL of the homogenous solution (from the previous step) to the fresh T75 tissue culture flask containing 14 mL of media.

Tissue culture flasks were labeled with name, cell type, passage number, and the date. It was stored in 37°C, 5% CO₂ incubator.
**Cell Preparation**

To prepare the cancer cells for the experiment, a volume of 1 mL or more of media from the 5 mL homogeneous solution (homogenous solution achieved from breaking the pellet with 5 mL of media) were transferred into a new conical using sterile instruments. The cell suspension in the conical tube were centrifuged for 3 minutes at 1.5G; a pellet should form in the bottom of the conical. The supernatant was aspirated from above the cell pellet without disturbing the pellet. It was then resuspended in 1 mL or more PBS+. Make sure to pipet up and down several times to get a homogenous solution. Make the according dilution to get an appropriate cell concentration to run through the functionalized channels.

**Cell Counting**

Cell counting was either performed with a hemocytometer or the Moxi Z automated cell counter using S cassettes (VWR). In order to use a hemocytometer for cell counting, 10 µL of cells were diluted in 10 µL trypan blue and 10µL PBS. Trypan blue is a vital stain that selectively colors dead cells blue because of their less intact membranes. The hemocytometer consists of a thick glass microscope slide with a rectangular indentation that forms a chamber on both sides. 10 µL of the mixture were loaded in each chamber. Cells were counted under an inverted light microscope (Olympus Microscope) in the area bounded by the lines. It was then possible to count the number of cells in an exact volume of fluid, and thus calculate the concentration of cells in the total fluid. The equation to calculate the cell concentrations was determined by the following equation:

\[
\frac{\text{number of cells counted (raw number)} \times \text{dilution factor} \times 10,000}{\text{number of squares counted}} = \frac{\text{cells}}{\text{mL}}
\]
The Moxi Z automated cell counter uses the Coulter Principle to provide a more accurate and repeatable cell count. The device contains a pre-filter to remove large cell aggregates and measure cell sizes ranging from 2 to 26 μm diameter. Thus, only single cells and possible small aggregates are counted. The counter outputs a histogram that shows the number of counts per cell diameter as well as calculate cell concentrations, mean cell diameter, and mean cell volume. The counter is set to have a specific gate to acquire data from a certain range of cell diameters. The counts were gated from 9.5 to 26 μm to disregard any dead cells or cell debris.

**Channel Surface Functionalization**

The antibodies were immobilized on the microfluidic channels prior to flowing cell samples through the devices. Before the microchannels undergo surface treatment, the fabricated herringbone device were sterilized under a UV lamp for at least 1 hour. To chemically modify the device in order to conjugate antibodies onto the surface of the channels, the microchannels were treated with 4% of 3-mercaptopropyl trimethoxysilane (Sigma Aldrich) in ethanol for 1 hour at room temperature, followed by rinsing with 100% ethanol. The microfluidic channels were then incubated in room temperature with 10 μg/mL malemide-activated NeutrAvidin (Life Technology) and Sulfo-SMCC (Fisher Scientific). NeutrAvidin is a deglycosylated version of avidin and has a high binding affinity towards biotin, the avidin-biotin interaction has the strongest non-covalent bond [22]. While sulfo-SMCC is a water-soluble heterobifunctional protein crosslinker. The devices can be stored in NeutrAvidin at 4 °C until use. On the day of the experiment, the channels were washed and incubated with 1% PBS+ for at least 1 hour at room temperature to
minimize any non-specific cell adhesion to channel surfaces. After incubation, 5 µg/mL capturing biotinylated antibodies solution in PBS+ were pipetted to the channels and incubated at room temperature for a minimum of 1 hour or more to allow the binding of biotin and NeutrAvidin molecules. Finally, PBS+ was used to rinse unbounded molecules after reaction and incubated again for 30 minutes in room temperature prior to running the experiment. A more detailed protocol can be found in Appendices. The four antibodies used for the experiments are anti-HER2 (R and D Systems), anti-EGFR (R and D Systems), anti-EpCAM (R and D Systems), and anti-MUC-1 (Fitzgerald). Cell lines corresponding high expression of these markers respectively are SK-OV-3, A431, H1650, and HeLa.

**Flow Cytometry**

Flow cytometry is a laser-based technology employed to obtain quantitative levels of biomarker expression. Cell components are fluorescently labeled and then excited by a laser as they flow past a laser beam to emit light at varying wavelengths. The forward detectors that follow the line of the laser capture forward scatter while perpendicular detectors to the laser capture side scatter. A detector also measures the fluorescence signals seen on each cell. This analysis can determine the levels of surface biomarker expression of the cells within a cell population. By looking at expression levels, decisions can be made on what cell lines are appropriate to use in different capture target experiments.

The cell samples were incubated with monoclonal antibodies specific to mucin 1 (MUC-1). The cell suspensions were centrifuged and resuspended in PBS+ containing 5 µg mL⁻¹ primary MUC-1 antibody (Fitzgerald). The samples were incubated for 30 minutes at
room temperature on a rotating platform. Following incubation, samples were washed twice with cold PBS+ and then resuspended in cold PBS+ containing 2 μg mL⁻¹ of a rat anti-mouse fluorescein-conjugated IgG1 antibody, acting as a secondary antibody. This solution was incubated for 30 minutes on ice, washed again three times with PBS+, and resuspended in .5 mL PBS+. In addition to the MUC-1 antibody conjugation, a separate sample was treated only with the FITC-conjugated IgG1 antibody to allow for measurement of background fluorescence. By using a LSR II flow cytometer and Flow JO software both cell scattering and fluorescence signals intensities were obtained. Normalized fluorescence intensity values were measured for each sample after subtracting background signals obtained with the control antibody to fluorescence signals.
3.4 Cell Testing through Functionalized Microchannels

Experimental Setup

Trypsinized cancer cells of interest spiked in PBS+ at known cell concentration was administered to the herringbone device using withdraw mode on the Harvard syringe pump. 15 mL conical held 5 mL of the cancer cells, which were attached to a vortex to allow the sample to be homogenous by continuously mixing during the experiment. Cancer cells were introduced to the channels at a flow rate of 2 µL/min for a fixed time (based on the concentration of the sample) to let 1,000 cells to flow through each channels. Two 1 mL syringes were connected to a 3-way valve at the outlet. One syringe served as a reservoir to collect the cancer cells that traveled through the
channel while the second syringe was preloaded with PBS+. After cells were withdrawn from the inlet to the reservoir, the valve was switched to PBS+. The buffer was passed forward for 10 minutes at 40 µL/min to flush any cells not adhered to the functionalized channels. The channels were then checked for any captured cancer cells by observing under the 10X inverted microscope (Hoffman Microscope).

**CTC Capture and Enumeration**

![Figure 19 Hoffman Inverted Microscope](image)

The capture of CTC was seen in the herringbone channels of all four different capture targets. The number of cells was counted after device processing of cells by manually counting under the inverted microscope. Since the number of cells introduced through the channel characterized at 1,000 cells for all the cell lines, the capture efficiency of the capture targets were calculated from the following equation:

\[
\text{Capture Efficiency (\%)} = \frac{\text{Final Cell Count Observed Under Microscope}(\text{cells})}{1,000 \ (\text{cells})} \times 100
\]
3.6 Results

Cell Capture

To test the effectivity of the antibodies’ functionalization, a very high cell count of 100,000 cells was flowed through the channel before lowering to a defined cell count to test the capture efficiency for the capture targets. To do this, we spiked a high cell concentration in PBS+ and ran at 2 μL/min through the experimental channel as well as the control channel where no antibodies were functionalized. Both channels were then washed with PBS+ at 40 μL/min for 10 minutes, so that any unbound cells were washed out of the channel. The control channel showed no CTC capture, which indicated that the antibodies-mediated cell adhesion played a major role in cell capture. Once we demonstrated that the conjugation of antibodies worked with the high cell count, we gradually lowered the cell count to 1,000 cells per channel in order to count the total number of cells that were captured inside the channel. Figure 20 displays a segment of the channel after flowing 1,000 and 10,000 SK-OV-3 cancer cells through a HER2 functionalized channel.
Capture Efficiency

For each of the four cancer cell lines, the capture efficiency was calculated as a percentage out of 1,000 cells at a flow rate 2 μL/min for its corresponding capture target. The average capture efficiency for the four capture targets is shown in Table 2 and Figure 21.

<table>
<thead>
<tr>
<th>Capture Target</th>
<th>Cancer Cell Line</th>
<th>Average Capture Efficiency (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>SK-OV-3</td>
<td>56.567</td>
<td>4.600</td>
</tr>
<tr>
<td>EGFR</td>
<td>A431</td>
<td>35.733</td>
<td>3.807</td>
</tr>
<tr>
<td>EpCAM</td>
<td>H1650</td>
<td>13.367</td>
<td>2.558</td>
</tr>
<tr>
<td>MUC-1</td>
<td>HeLa</td>
<td>8.067</td>
<td>3.256</td>
</tr>
</tbody>
</table>

Figure 20 Cells Captured on Herringbone CTC Capture Device

The capture of cells after flowing through A) 1,000 and B) 10,000 SK-OV-3 cancer cells through the device.
A control channel was run in parallel to the experimental channel. The control confirmed no cell adhesion to the surfaces of the channel. A 3-way valve was used to open the PBS+ reservoir to flush PBS+ after the cell run to prevent mechanical disturbance from reinserting tubing. This might have caused cells to dislodge from the adhered channel otherwise. This resulted in obtaining higher average capture efficiency.

**Biomarker Expression**

In order to choose a suitable cell line for MUC-1 capture target, we sought to measure MUC-1 expression via flow cytometry. The cancer cell lines corresponding to the highest expression shown in Figure 22 were H1650 and HeLa. H1650 had 3-fold higher expression of MUC-1 than SK-OV-3 while the expression of HeLa was 2-fold higher than SK-OV-3. A431 had a significantly lower expression of MUC-1.

![Average Capture Efficiency (%) for Capture Target](image)

*Figure 21 Average Capture Efficiency for Capture Targets*

The panel shows sample of 1000 cells run through the device at 2 µL/min. Samples counted after device was flushed with PBS+ at 40 µL/min for 10 minutes.
3.7 Discussion

Characteristics of the device and channels were made to test the CTC capture device. The Toner lab established an optimal flow rate for the device—2 µL/min flow rate—that we used as the optimal rate for our following experiments. The functionalization of the channels through avidin-biotin chemistry was essential for cell adhesion to the surface of the channel. We observed cancer cells captured on the herringbone channels from a high cell count to a low cell count. The capture efficiency was calculated for 1,000 cell count through the channel.

We obtained high capture efficiency for most of the capture targets. HER2 targeted ligand gave a relative high count compared to the other markers. Meanwhile EpCAM gave a low average capture efficiency compared to the basis reported from Toner Lab [19]. Capture efficiency is limited by the antibodies and its ability to bind to the targeted ligands.
This discrepancy can be caused by the antibodies provided by R&D systems that may have lost protein expression. It is possible to resolve this by finding a suitable monoclonal antibody instead of a polyclonal antibody thereby increasing the binding efficiency of the antibodies for their respective ligands. EpCAM targeted ligand can further be tested from a different vendor in order to confirm an accurate cell capture.

Lastly, we implemented flow cytometry to analyze the expression level of MUC-1 in order to choose an appropriate cell line for the capture experiments. MUC-1 was the only monoclonal capture target used because the monoclonal form is underglycosylated. From the results obtained from the biomarker expression, we decided to use HeLa cell lines for our MUC-1 capture run. However, H1650 cell line can yield a higher cell capture since it has a higher expression of MUC-1 compared to HeLa. These results allowed us to move forward and helped the transition into a multi-marker capture channel device.
CHAPTER 4

CONCLUSION

4.1 Concluding Remarks

The microfluidic device to capture circulating tumor cells presented in this thesis has the potential to be used as both a diagnostic and prognostic tool. The initial design of a microfluidic device originated from my senior design project where we focused on creating an inexpensive, semi-automated, and high throughput detection system. After two iterations of a CD device, we decided to focus on achieving a higher capture efficiency by using a peristaltic pump to flow cancer cells through a single channel. We were able to demonstrate capture efficiency with different capture targets, which enabled us to conduct future work using a single device that incorporates multiple capture targets.

4.2 Future Work

To obtain a higher capture efficiency, we will continue testing the capture targets with the Toner chip (Figure 23). This will be accomplished since the design has a higher surface area since it has four channels that converge from inlet and diverge to the outlet. We were able to achieve an almost 3-fold increase with MUC-1 capture target of 22% capture efficiency with the Toner chip compared to 8%. After we confirm our anticipated results of a higher capture efficiency for the other capture targets, we will implement a multi-capture CTC device.
The CTC capture device provided the foundation to make a fully integrated multi-capture CTC device. Our future work will be to test the four capture targets in series to determine total capture efficiency in the four different channels. Additionally, the cells captured will be sorted based on the antibody marker they are attached to which can be directly analyzed on the chip. However, since the surface area is decreased by a factor of one fourth for each biomarker, the flow rate must be lowered to increase the throughput to ultimately offset the otherwise predicted drop in capture efficiency. In essence, it will be possible to gain a higher capture efficiency and to prescreen the cancer profile based on surface expressed marker.

A herringbone multi-capture device was designed to have four separate channels using SolidWorks similar to the design of the herringbone chip [19]. However, the device was modified to include separate inlets and outlets for each channel to enable parallel operations and as well as allowing connections to each channels in a sequential order as shown in Figure 24. Fluid flow direction is controlled using integrated valves. These four parallel channels on the device can be conjugated with different antibodies so that cells of interest can be captured in a sequential manner. Once different antibodies are conjugated
in separate channels, the cells can travel through the entire device in a serpentine flow pattern.

We will perform the same set of experiments as with the previous device. We will start out by coating a single channel to obtain capture efficiency for its corresponding capture targets. After the capture efficiencies are determined for a single capture in the multi-channel device, we will then functionalize each channel separately with different makers. The first channel will be coated with anti-EpCAM, the second channel will be anti-HER2, the third channel will be anti-EGFR, and the last channel will be anti-MUC-1 displayed in Figure 25. The cell lines that we will use for this study will be H1650, SK-OV-3, A549, and HeLa. These cell lines were chosen based on their high expression of corresponding capture target while having low to medium expression of the other targets [15]. This will allow us to sort the cells depending on its corresponding target expression. For example, cells expressing higher expression of EpCAM will mostly be captured in the

![Figure 24 Multi-Capture CTC Device](image)

A) Different antibodies will be functionalized in four parallel channels with separate inlets and outlets. B) Cell capturing will be achieved with all tubing connected as illustrated to allow channels to operate in series.
first channel. Any cells not adhered in the first channel will continue traveling through the other channels to potentially get captured with a different capture target.

This application will lead to a higher capture efficiency by allowing multiple capture targets to be employed during the experimental run. It will be helpful to conduct further help to further studies on the cells depending on which capture channel the cells are captured on.

![Capture Targets for CTC Multi-Capture Device](image)

**Figure 25 Capture Targets for CTC Multi-Capture Device**

Each parallel channel is functionalized to a different capture target. Channel 1 will be coated with anti-EpCAM, channel 2 will be coated with anti-HER2, channel 3 will be coated with anti-EGFR, and channel 4 will be coated with MUC1.
REFERENCES


Appendix

Standard Operating Procedures

Preparing to reconstitute Human EpCAM/TRP-1 Biotinylated Antibody Protocol

R&D Systems

**Materials**
50 ug of human EpCAM/TROP-1 Biotinylated Antibody
250 ul PBS+
Micro-centrifuge tube

**Methods**
1. Add 150 uL sterile PBS to vial containing 50 ug of EGFR to make .2 mg/ml of Biotinylated EpCAM.
2. Vortex to mix the solution
Preparing to reconstitute Human ErB2/Her2 Biotinylated Affinity Antibody Protocol

R&D Systems

**Materials**
- 50 ug of human Human ErB2/Her2 Biotinylated Antibody
- 250 ul PBS+
- Microcentrifuge tube

**Methods**
1. Add 150 uL sterile PBS to vial containing 50 ug of Her2 to make .2 mg/ml of Biotinylated EpCAM.
2. Vortex to mix the solution
Preparing to reconstitute Human EGFR/ErbB1 Biotinylated Antibody Protocol

R&D Systems

**Materials**
50 ug of human Human EGFR/ErbB1 Biotinylated Antibody
250 ul PBS+
Microcentrifuge tube

**Methods**
1. Add 150 uL sterile PBS to vial containing 50 ug of EGFR to make .2 mg/ml of Biotinylated EpCAM.
2. Vortex to mix the solution
Biotinylate MUC-1 Antibodies Protocol

Fitzgerald
Thermo Fisher

**Materials**
- 25.77 uL of primary Muc-1 Antibody
- 423.76 uL sterile PBS
- .46 uL NHS-Biotin
- 50 uL Sodium Bicarbonate
- Zeba Column
- Microcentrifuge tube

**Methods**
1. In a 1.5 mL centrifuge tube, add PBS, Sodium Bicarbonate, and Primary Antibody. Vortex.
2. Add biotin to reaction solution. Vortex.
3. Incubate at room temperature for hours on gyromini
4. To exchange buffer in Zeba Column, add 1 ML PBS slowly over column bed.
5. Centrifuge at 1000 x g for two minutes
6. Repeat steps 4-5 two times
7. Transfer Zeba Columns to a fresh 15 mL conical tube
8. Drip reaction mixture slowly over the column bed.
9. Once the entire sample has entered the column bed, centrifuge at 1000 x g for 2 minutes
10. Transfer product to 1.5 mL centrifuge tube.
11. Obtain sample concentration using Nanodrop
12. Store at 4 °C.
Preparing 4% 3-Mercaptopropyl Trimethoxysilane Protocol

Sigma Aldrich

**Materials**
600 ul silane
15 mL ethanol
Pipette-man
Pipette aid
25 mL serological
Sharps chemical waste container
50 mL centrifuge tube

**Methods**
1. Label 50 mL centrifuge tube with the following:
   - 4% Silane
   - Date
   - TOXIC (stored only in chemical hood)
2. Pipette 15 mL of ethanol to centrifuge tube
3. Pipette 600 uL silane into 15 mL ethanol. The final diluted stock will produce 15 mL. The final diluted stock will produce 15.600 mL of 4 % silane.
4. Secure cap to bottle before cleaning hood. Final diluted stock should be kept inside the fume hood at all time
Preparing 10 ug/mL Malemide-Activated NeutrAvidin Protocol

Sigma Aldrich

**Materials**
2mg no-weigh sulfo-SMCC  
200 uL DMF  
200 ul neutravidin (250 ug/ml in PBS)  
750 PBS +400 ul PBS  
Pipette-man  
2 mL Zeba column  
Microcentrifuge tube (Eppendorf tube)  
15 mL centrifuge tube

**Methods**
1. Label Eppendorf tube with the following:  
   ✓ Sulfo-SMCC  
   ✓ Sulfo-SMCC+Neutravidin:maleimide-activated neutravidin  
   ✓ Date
2. Reconstitute 2 mg no-weigh sulfo-SMCC (-20 C) in 200 ul DMF (flammable cabinet). Store unused in -80 C.
3. Add 50 uL sulfo-SMCC to 200 uL NeutrAvidin (250 ug/mL in PBS) into an eppendorf tube. Incubate at RT for 1 hour.
4. Centrifuge 2 mL zeba column at 1000g for 3 mins to remove storing solution.
5. Add 750 uL PBS to maleimide-activated NeutrAvidin to a total of 1 mL. Add everything to Zeba column, centrifuge at 1000g for 3 mins. Store unused at -80 C.
6. Make aliquots of 100 uL of the maleimide-activated NeutrAvidin and keep in freezer until used.
7. Once ready to use, add 400 ul PBS+ to 100 uL maleimide-activated NeutrAvidin to a total of 500 uL, 10 ug/mL maleimide-activated NeutrAvidin in micro-centrifuge tube.
Immobilizing Antibodies on the Channel Surface Protocol

Sigma Aldrich

Materials
3x1” glass slide 200 uL DMF
100% EtOH
Device
Pipet

Methods
1. Sonicate 3x1” glass slide in 100% EtOH for 10m, dry with N\textsubscript{2} gun.
2. Plasma treat glass slide with PDMS
3. Prior to modify the device surface, keep the devices in UV for 15-30 mins.
4. Incubate device in 4% 3-mercaptopropyl trimethoxsilane at RT for 1 hour
5. While waiting, prepare 10 ug/mL maleimide-activated neutravidin.
6. After 1hr silanization, rinse channels with 100% ethanol at least 3x, dry with N\textsubscript{2}.
7. Bake device dry at 70°C for 30-45mins.
8. Pipet (~75ul/channel) of 10 ug/ml maleimide-activated NeutrAvidin and incubate @RT for 1 hr to attach neutravidin to sulfo-SMCC.
9. Store devices in neutravidin at 4°C up to 2 weeks (or until use)
10. Wash the chamber with 1% BSA (PBS+). Block with 1% BSA at RT for 1 h.
11. Aspirate out the PBS+ in the channel and dry with oxygen gas.
12. Incubate with at least 75 ul of 5ug/ml capture antibody per channel @RT for minimum of 1h
   - EpCAM: (dilution to get 75 ul per channel 5 ug/ml)
   - HER2: (dilution to get 75ul per channel 5 ug/ml)
   - EGFR: (dilution to get 75ul per channel 5 ug/ml)
   - MUC-1: (dilution to get 75ul per channel 5 ug/ml)
13. Wash with 1% BSA (PBS+). Incubate with 1% BSA(PBS+) at RT for 30mins (can store in fridge for up to 1 hr, should be immediately used).
Photomask for Toner Chip and Multi-Capture CTC Device

A

Mask 1

B

Mask 2