

# POINT OF CARE ANEMIA DEVICE

BME 400 Final Report

December 11, 2015

Team Members:

Carly Hildebrandt – Team Leader

Katie Barlow – Communicator

Jeff Wu – BPAG

Lizzie Krasteva – BSAC

Michal Adamski - BWIG

Advisor: Dr. Thomas Yen

Client: Dr. Philip Bain

## Abstract

Anemia affects twenty-five percent of the global population, being most prevalent in the underdeveloped countries of Africa. Most types of anemia are preventable, but lack of funding and resources for complete blood tests prevents clinicians from making a proper diagnosis and suggesting appropriate treatment. The goal is to develop a cost-efficient, portable, and accurate device to count red blood cells and measure their mean corpuscular volume. A previous team developed a proof-of concept using a microfluidics channel and a computer interface to measure the resistance change of microparticles moving through the microchannel. There are many improvements to be made on the device, and the focus will be on pumping and filtering methods. Each design was evaluated using a design matrix, and the team decided to move forward with the syringe pump and cell filters. Cell filters were testing and statistical analysis showed significant differences before and after filtering meaning more than white blood cells were lost in the process. Active and passive pumping techniques were evaluated using diluted porcine blood samples to determine the microchannel's ability to count cells. The fluctuations of the channel resistance during active pumping led to inconclusive results and while passive pumping showed a few peaks correlating to cells, not enough were counted to make any claims at this time.

## Table of Contents

<b>Introduction .....</b>	<b>4</b>
<i>Motivation.....</i>	<i>4</i>
<i>Problem Statement .....</i>	<i>4</i>
<i>Past Work.....</i>	<i>4</i>
<i>Competing Designs.....</i>	<i>6</i>
<b>Design Alternatives .....</b>	<b>7</b>
<i>Pumping Techniques .....</i>	<i>7</i>
<i>Filtering Techniques .....</i>	<i>9</i>
<b>Design Evaluation.....</b>	<b>12</b>
<i>Design Matrices.....</i>	<i>12</i>
<i>Proposed Final Design .....</i>	<i>14</i>
<b>Fabrication/Development Process.....</b>	<b>15</b>
<i>Materials .....</i>	<i>15</i>
<i>Methods .....</i>	<i>15</i>
<i>Final Prototype .....</i>	<i>15</i>
<i>Testing.....</i>	<i>16</i>
<b>Results .....</b>	<b>18</b>
<i>Filtration.....</i>	<i>18</i>
<i>Pumping Data Collection .....</i>	<i>19</i>
<b>Discussion .....</b>	<b>21</b>
<i>Ethics .....</i>	<i>21</i>
<i>Necessary Changes.....</i>	<i>21</i>
<i>Sources of Error .....</i>	<i>22</i>
<b>Conclusions .....</b>	<b>22</b>
<i>Future work .....</i>	<i>23</i>
<b>References .....</b>	<b>25</b>
<b>Appendix.....</b>	<b>i</b>
<i>Data Analysis.....</i>	<i>iii</i>
<i>Materials .....</i>	<i>v</i>

## Introduction

### Motivation

Anemia is a deficiency of the hemoglobin in the blood, usually characterized by abnormal size, shape, and reduced number of red blood cells and quantified by measuring hemoglobin and mean corpuscular volume. Common symptoms of anemia are fatigue, dizziness, rapid heartbeat, and shortness of breath. [1] There are many types of anemia, but the team's focus is microcytic, normocytic, and macrocytic anemia. Microcytic is characterized by abnormally small red blood cells, usually in the range of 4 to 6  $\mu\text{m}$  in diameter and less than 80 fL volume, and is caused by iron deficiency. Normocytic is characterized by having normal-sized red blood cells (6-8  $\mu\text{m}$ , 80-100 fL) but is caused by decreased production or increased destruction of red blood cells, such as hemolysis. Finally, macrocytic is characterized by abnormally large red blood cells, usually in the range of 8-10  $\mu\text{m}$  in diameter and greater than 100 fL volume, and it is caused by vitamin B12 deficiency or hypothyroidism. [2]

Anemia is very prevalent in underdeveloped countries, due most commonly to malnutrition. Anemia affects roughly twenty-five percent of the global population, but the highest prevalence is in Africa, making up about sixty percent of those globally affected by anemia. Most types of anemia are not only treatable but can be prevented. However, lack of funding and resources for complete blood tests in these developing countries block the ability for clinicians to properly diagnose anemia and suggest treatment. [3] The client, Dr. Philip Bain, volunteers with Global Brigades, an international non-profit organization that helps communities around the world meet their healthcare and economic needs. [4] After volunteering in Ghana, Africa, he wanted to initiate the development of an inexpensive anemia detection device to allow clinicians to make a proper diagnosis and give appropriate treatment options.

### Problem Statement

Anemia affects many people worldwide and disproportionately affects those in developing countries due to a lack in medical infrastructure to properly diagnose the blood disorder. A portable, easy to use, and cost-effective device is needed to diagnose the condition in these countries at the time of initial medical care. Anemia can be diagnosed by evaluating red blood cell size using the mean corpuscular volume (MCV). The goal is to fabricate a microfluidic device that effectively measures the MCV of red blood cells to determine if a patient has normocytic, macrocytic, or microcytic anemia with results comparable to current cell counting techniques.

### Past Work

Last year, a team developed a proof-of-concept device for this project, consisting of a microfluidics channel and computer interface using LabVIEW. They used a passive pumping method in one inlet-outlet pair to transport 10  $\mu\text{m}$  microparticles through a microchannel, and then they measured the resistance change across the other inlet-outlet pair (See figures 1,2). Each peak in figure 3 represented a microparticle moving from the left side of the channel to the right, while the height of the peak determined the particle's relative size.

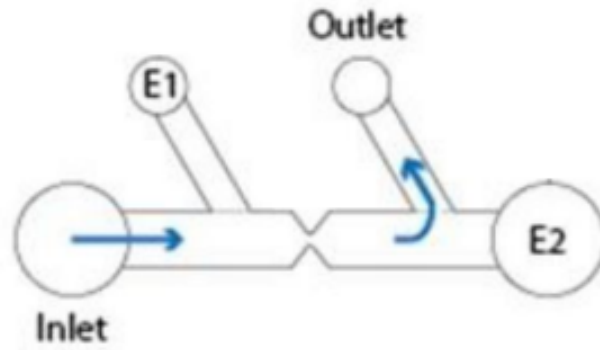


Figure 1: Channel design. The inlet and outlet represent the flow of the microparticles through the channel, and E1 and E2 represent the electrodes. [5]

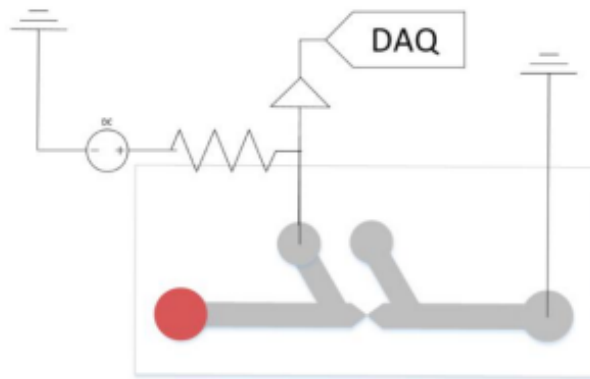


Figure 2: Circuit schematic of the electrodes that measure the resistance change across the microchannel. [5]

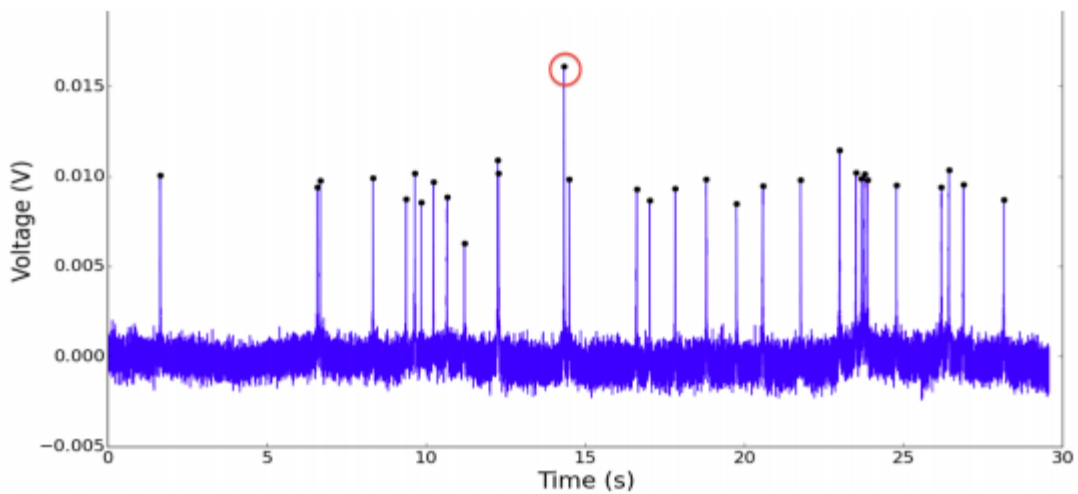


Figure 3: Voltage vs time with coincident event of a microparticle moving through the microchannel. [5]

There is a lot of work left to be done for the device, however. A meeting with the previous team was arranged where potential improvements on their prototype were discussed. They mentioned the difficulty of passive pumping, as adding the proper amount of solution to both the inlet and the outlet for the passive pumping to work is tedious. In addition, they never were able to test whole blood for the device, and a filter is needed for the device to remove unwanted blood cells, such as white blood cells and platelets.

## Competing Designs

In addition to researching the past work of the previous teams, we also looked into other competing designs already available. The two designs closest to what we are trying to accomplish are the Coulter counter and the Lab on a Chip Blood Counting Device. Both devices would work very well in theory for solving the current problem, but also offer significant disadvantages to the current work that has been done.

### Coulter Counter

The Coulter counter offers many hospitals and clinics the ability to count and size various cells and particles in an electrolyte solution. [6] The advantages of this device are that whole blood can be used, and it is versatile in that it can be used for more counting strategies than simply determining anemia. Despite being advantageous in allowing many counting strategies, this is also somewhat of a disadvantage when compared to our ideal device because it is a bit more complex than what we need. The device does also come at a significant cost - most Coulter counters are on the order of \$2,000 or more, and we are looking for a device that can be bought in an underdeveloped country for around \$50. In addition, the Coulter counter is a bit large, therefore making it more difficult to transport.



Figure 4: Coulter counter [7]

### Lab on a Chip - On-chip sample preparation for complete blood count from raw blood

The second device we found was created at the University of Toronto in Toronto, ON, CA. The device can also use whole blood and works by filtering white blood cells from red blood cells, then diluting, lysing, and measuring the cells in count and size. [8] The device is much smaller than the Coulter counter, as it is a microfluidic device made of PDMS and is the size of a microscope slide, making the device very portable (see Figure 5). The device also uses a pneumatic pressure device to drive the

fluids through the various channels, which is easier to use than the passive pumping technique the previous team used for our device. However, the device is still more complicated than what we need, since we are only interested in red blood cell count and size, and the pneumatic pressure device to drive the fluid through is costly in itself, making the overall cost of the device on the order of \$500. Therefore, we decided to continue on where the previous design team left off on our device and make the necessary changes needed.

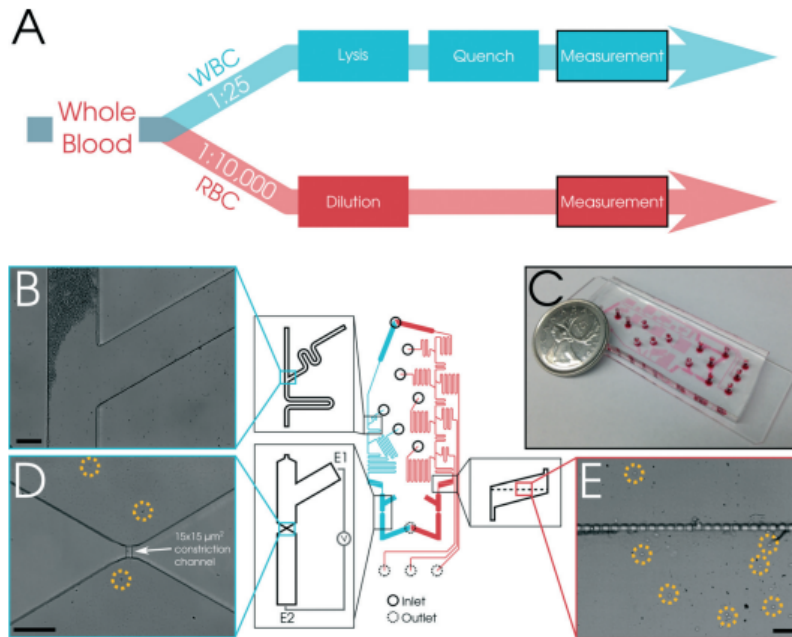


Figure 5: On-chip sample preparation for complete blood count from raw blood [8]

## Design Alternatives

In order to continue with the prototype and design of the previous team, two components were evaluated for integration into the design. While the previous team proved the effectiveness of a microfluidic counting channel, blood was never analyzed with the device. Due to blood's heterogeneous composition and high cell concentration, it must go through a preparation process prior to being sent through the channel. This involves filtering the sample, so only red blood cells cross the aperture, and diluting to a low concentration so that only one cell passes through the channel at a time. The two design components that were evaluated were pumping and filtering techniques.

## Pumping Techniques

The pumping method is necessary to evaluate the best technique to pass the blood sample through the device quickly and effectively.

### Passive Pump

The passive pumping technique works by allowing the cells to travel through the channels down their concentration gradient. The counting channel would be filled with PBS or some similar conducting solution. When a droplet of a diluted blood solution is placed on one end of the channel the solution will

spread and move to the opposite side of the channel simply because it wants to travel from high concentration to low. An overview of this process can be seen in figure 6.

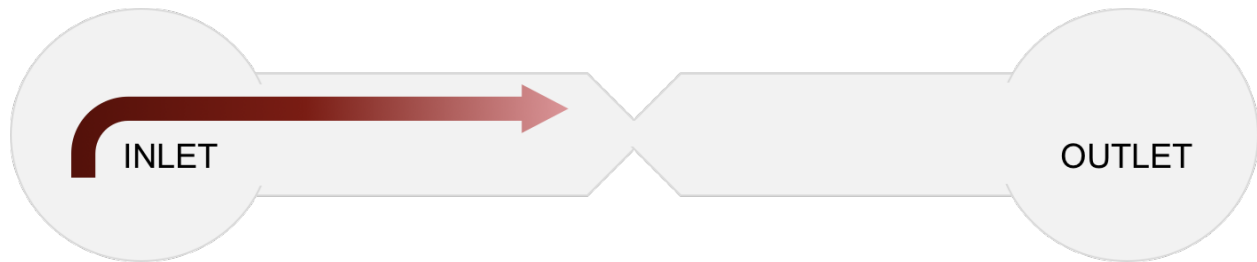


Figure 6: Overview of passive pumping technique. Droplet of blood solution is added to inlet end of channel and blood cells will flow towards the outlet end, down their concentration gradient.

This technique requires very little additional materials. A pipette would be needed to add a droplet of known volume to the channel and that is all. This allows this design to be very low cost per use. There are quite a few disadvantages that accompany this design. It is very difficult to place a droplet on such a small surface and requires a skilled user. This increase in user difficulty can hinder the time it takes to run the diagnostic test and increases the risk in wasting materials if the clinician is more prone to mistakes. There is also very little control over the rate of flow of the cells through the channel. Additionally, this can increase usage times and decrease the amount of tests that can be performed in some allotted amount of time. Also since there is not any active pumping taking place, the direction of flow might not be unidirectional which would greatly skew results.

### Syringe Pump

Using a syringe to manually pass the blood sample through the device is another pumping option. This design includes a small syringe filled with the diluted blood solution and a tubing channel that connects to the microfluidic counting channel. The user would manually compress the syringe to send the sample slowly through the sample. The rate of flow could be measured based on the speed at which the user compressed the syringe and the diameter of the syringe opening where cells will be leaving and entering the channel. The set-up to using a syringe as the mode of pumping blood through the channel can be seen in figure 7.

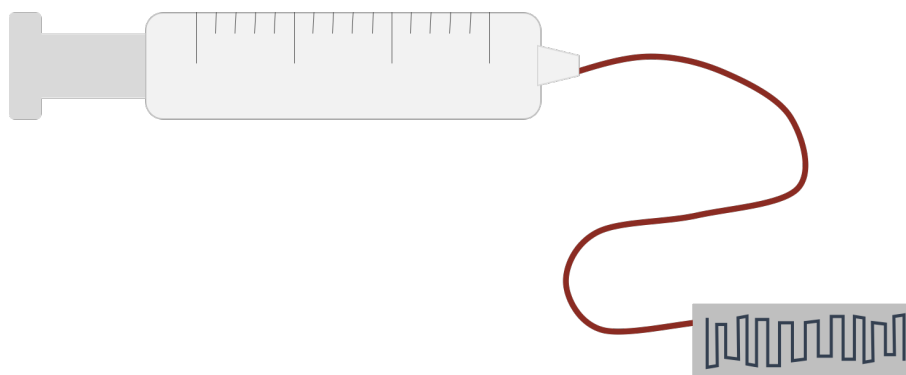




Figure 7: Syringe attached to a microfluidic channel. User would manually compress syringe to send blood sample through the channel. The syringe would be integrated into the channel through an additional tube as shown.

Some advantages of this design is the overall simplicity and ease of fabrication. All components of this pump can be bought commercially and easily integrated into the existing device. Since many clinicians are familiar with operating a syringe, it should also be simple to use. The disadvantages include its tendency for variation between each test. Because the flow rate depends much on the user and the speed at which the user compresses the syringe, this may cause differences in flow between samples. The user would need to apply a continuous pressure at a specified speed to perform the diagnostic test correctly.

### Peristaltic Pump

A peristaltic pump to send the blood sample through the channel is a common microfluidic technique and another design alternative that was considered. This pump incorporates flexible tubing and a rotor to send the blood solution through. The rotor compresses the tubing as it rotates pushing fluid through the tubing unevenly. A peristaltic pump similar to what would be integrated into our design is shown in figure 8.

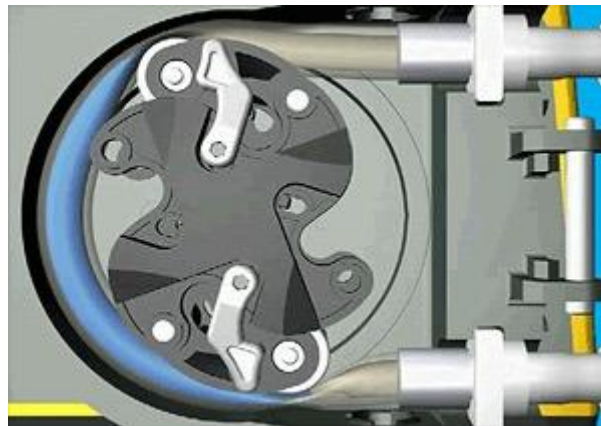


Figure 8: The peristaltic pump tubing and rotor shown here. The rotor rotates, compressing the tubing and sending the fluid (which can be seen in blue) through the channel. [9]

The advantages of using this type of mechanical pump is its automation and ease of use. The pump only needs the user to load the blood sample and the pump will automatically send the fluid through the channel. Because of this automation, the flow rate will also be consistent throughout each test and have no dependency on the user. Because of the pumps mechanical properties and variety of moving parts, this design will require more maintenance than the other options. For use in developing countries where resources are limited, this can be very problematic.

### Filtering Techniques

Anemia can be diagnosed by analyzing red blood cell volume and size. Blood is made up of more components than just the desired red blood cells and in order to get the most accurate measurement, a filtering technique is necessary to prepare the sample so that only red blood cells remain. With a

filtering mechanism, the goal is to have only RBCs sent through the aperture of the microfluidic counting channel.

### Cell Filter

After the cells are delivered into the device via a pumping mechanism, they must be filtered to isolate the red blood cells from the other components of blood. One option is a syringe filter, shown in Figure 9, which contains mixed cellulose ester filter membranes in plastic housings. [10] This filter would be attached to the pump, and cell solution would be pumped through the filter before it is sent through the microfluidic portion of the device for analysis. The filter pore size would be about  $11\ \mu\text{m}$  in order to exclude white blood cells, which have a diameter of  $12\text{-}15\ \mu\text{m}$ , and enable entry of red blood cells, which have a diameter between  $4\text{-}10\ \mu\text{m}$ . The wide range of red blood cell sizes reflects the type of anemia, where microcytic anemia is displayed by an RBC diameter closer to  $4\ \mu\text{m}$ , and macrocytic anemia is manifested by a diameter closer to  $10\ \mu\text{m}$ . The diameter of platelets is on the order of  $1\text{-}3\ \mu\text{m}$ , so they will pass through the filter, but the analysis is not presumed to be affected by their presence.



Figure 9: A syringe filter is connected to the pump and allows for cell solution to be pumped through the filter, removing white blood cells. [10]

This single syringe filter would be easy to manufacture since the filter units are available on the market, and they would only need to be adapted to the pump and microfluidic chip portions of the design using a simple method such as attaching the pump outflow to the filter using a tube and the filter outflow to the chip using another tube. Operation time would be low with this design since the cell solution would only be passing through a single filter. However, this filter would have short lifespan since it is not reusable. In developing countries with few resources, it may be unfeasible to replenish stocks of filters on a regular basis.

### Cascading Filter

Another filter option is a multiple-layered cascading filter that would be made up of three filters of decreasing pore size. The filter would be the size of a 5 mL syringe and contain three disks made of polyethylene secured to the walls of the cylindrical syringe, as shown in figure 10. These three disks that contain perforations will allow for successive movement of red blood cells through based on diameter. At the top layer, all but the largest cells (WBCs of  $\sim 15\ \mu\text{m}$  diameter) will be allowed to flow through to the layer underneath, and the layer underneath will contain smaller pores that exclude cells of a  $13\ \mu\text{m}$

diameter. The final layer would block passage of cells under  $11\ \mu\text{m}$  wide, effectively isolating red blood cells from white.

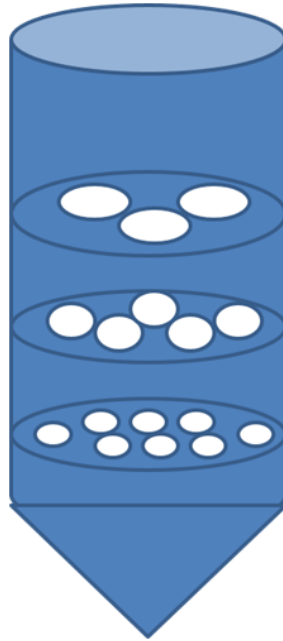


Figure 10: A cascading filter isolates red blood cells by removing larger white blood cells from the cell solution using a succession of three layers, each containing a smaller pore size than the one above.

This design would decrease cell accumulation within the filter by having multiple layers which allow cells to flow gradually through the device instead of building up and clustering together above the filter. However, the design would be difficult to fabricate since this type of filter is not currently available on the market and would have to be built by the design team. In addition, a longer operation time would be necessary with this filter since the cell solution would have to pass through multiple filters instead of just one filter as in the case of the aforementioned cell filter.

### **Built-in Microfluidic Filter**

A final option for the filter is a built-in microfluidic filter that would be incorporated into the microfluidic chip apparatus used for cell analysis built by the previous design team. As shown in figure 11, a row of pillars with  $11\ \mu\text{m}$  openings would be constructed on the chip to allow red blood cells to pass through the openings while blocking passage of the white blood cells, which are larger.

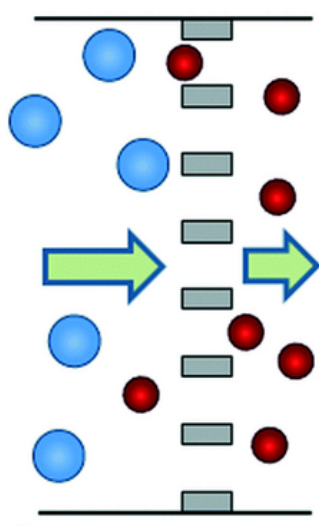


Figure 11: A built-in microfluidic filter consists of channels that allow for passage of red blood cells into the microfluidic device and exclusion of white blood cells. [11]

This built-in filter would be easy to use since it is integrated into the existing device and would allow filtration to be accomplished without using a separate physical component. On the other hand, the filter itself will have to be made on the microscale, suggesting that fabrication will be difficult since much smaller parts are involved. Additionally, white blood cells and debris may clog the filter quickly since there are only a certain number of openings to allow for cells to pass through.

## Design Evaluation

Pumping and filtering techniques were analyzed and evaluated based on a variety of categories in order to propose the most effective final design.

### Design Matrices

Design Criteria	Passive Pump		Syringe		Peristaltic Pump	
<b>Ease of Use (25)</b>	1/5	5	4/5	20	2/5	10
<b>Time of Use (25)</b>	1/5	5	3/5	15	3/5	15
<b>Cost (20)</b>	5/5	20	3/5	12	2/5	8
<b>Ease of Manufacturing (15)</b>	5/5	15	4/5	12	3/5	9
<b>Size (10)</b>	5/5	10	3/5	6	2/5	4
<b>Safety (5)</b>	5/5	5	5/5	5	5/5	5
<b>Total (100)</b>	<b>60</b>		<b>70</b> ★		<b>51</b>	

Figure 12: Design matrix for alternative pumping techniques.

A design matrix was created for the three aforementioned pumping techniques (Figure 12). The designs were ranked on the six following categories in order of decreasing weight: ease of use, time of use, cost, ease of manufacturing, size, and safety.

Ease of use is of high priority to our client, Dr. Bain. He said that the experience level of clinicians at point of care clinics in developing countries varies. He wanted our team to design a device that would allow for clinicians of any skill level to use. The passive pumping technique is a difficult procedure to set up, and clinicians who have never used a passive pumping technique before would need to be trained to perform this technique. Although a peristaltic pump is convenient because it can regulate the flow rate, this piece of equipment is expensive and bulky. Therefore, the syringe scored highest for ease of use. Clinicians of varying skill levels have most likely used a syringe before, either for drawing blood or administering shots. They are already familiar with this piece of apparatus, so they would not need any additional training to utilize our device.

Time of use is also of high priority to our client and was weighted the same as ease of use. Dr. Bain said point of care clinics see about 300-500 patients per day, so our device needs to be quick to use. The syringe and peristaltic pump tied in this category, and the passive pump scored the lowest. The previous design team said that it took them a few tries to get the passive pump to work, so that is why it scored the lowest in this category.

The cost category was determined by additional costs to the current device. The passive pumping inlets and outlets are already built into the current device, so no changes need to be made to the current design. The syringe and peristaltic pumping techniques would require additional equipment to be purchased. This caused these two techniques to score lower in this category, with the peristaltic pump scoring lowest because it's significantly more expensive than the other two design alternatives.

Ease of manufacturing was based on how much additional work would be needed to adapt the pumping techniques to the current design. Again, since passive pumping is already part of the current design, it scored highest in this category. The syringe scored second highest because the inlets of the current design would have to be modified to fit the syringe. The peristaltic pump scored the lowest because in addition to modifying the inlets of the current design, the other end of the peristaltic pump would have to be fitted to the blood sample container.

Size was based off of the current device. Therefore, the passive pump scored highest. The syringe scored second highest because it's an additional piece of equipment that needs to be integrated into the current design. The peristaltic pump scored lowest because it is a bulky piece of equipment that is much larger than the microfluidic device.

Safety was not a concern for the three different designs. Clinicians who work in clinics are trained in handling bodily fluids. The three proposed designs do not expose the clinicians to anything they are not already exposed to.

Design Criteria	Cell Filter		Cascading Filter		Built-in Filter	
Ease of Use (25)	4/5	20	4/5	20	5/5	25
Time of Use (20)	3/5	12	2/5	8	4/5	16
Cost (20)	5/5	20	2/5	8	2/5	8
Ease of manufacturing (15)	5/5	15	3/5	9	1/5	3
Size (10)	4/5	8	3/5	6	5/5	10
Lifespan (5)	2/5	2	3/5	3	3/5	3
Safety (5)	5/5	5	5/5	5	5/5	5
<b>Total (100)</b>	<b>82</b> ★		<b>59</b>		<b>70</b>	

Figure 13. Design matrix for alternative filtering techniques.

A second design matrix was created for the three filtering techniques (Figure 13). The designs were ranked on the seven following categories in order of decreasing weight: ease of use, time of use, cost, ease of manufacturing, size, lifespan, and safety.

Ease of use and time of use follow the same criteria as mentioned in the pumping techniques section. The built-in filter scored highest in these two sections because the filtering component is incorporated into the existing design and no additional equipment is needed. The cell filter scored highest for cost and ease of manufacturing. Cell filters are readily available and can be purchased in bulk at a low cost. Although microfluidic devices are fairly inexpensive to manufacture, the research and design needed to get to a functional prototype can be quite expensive. Masks needed to fabricate the microfluidics devices can cost over \$100 per mask, and it's not guaranteed that our microfluidic filter design would work. The built-in filter will be integrated into the existing microfluidic, so it scored highest in the size category. The cascading filter and built-in filter tied for lifespan because these two designs are reusable. Safety was also not a concern for these techniques as the clinicians should be trained to properly handle bodily fluids.

### Proposed Final Design

As seen from the design matrices, the syringe and cell filter scored the highest and will be incorporated into the final design. The familiarity that clinicians have with syringes was a big factor in choosing this design. In addition, syringes are readily available and of low cost. Similarly, cell filters are also readily available and of low cost. Although cell filters are not reusable like the cascading and built-in filter, our team felt that developing countries lack the necessary resources and time to keep the reusable filters cleaned and maintained. Thus, the cheaper and easier alternative is to go with the disposable cell filter.

## Fabrication/Development Process

### Materials

For this semester's work, filtering was determined to be a major area of focus. For this part of the design, a sample pack of variously sized, round filters was purchased. These filters have a pore size of about 10 microns; this was determined to be an adequate size to let red blood cells through while at the same time trapping the white blood cells in the collected samples.

PDMS was the material of choice for the fabrication of the microchannels used in this project. The previous group that worked on this project used PDMS with general success and a substantial amount remained from their work. Therefore, it was used again for the continuation of this project.

Syringe to channel attachment required a series of Luer locks and tubing. These were secured to the PDMS channel inlet port with a strong clear adhesive to prevent leakage and backflow.

### Methods

The channel itself was molded from PDMS, vacuumed and allowed to set over the master disc designed by the previous team. It was fabricated so that the thickness was approximately 1cm high. After the PDMS had cured, the channel was removed from the master disc and plasma treated to a glass slide. The channel design contained four ports, two for electrode placement, an inlet and an outlet. All four were punched from the PDMS using a biopsy punch. A Luer lock adapter was placed into the inlet port, where it was securely sealed with a strong adhesive. The micro-scale size of the aperture requires a strong seal at the inlet port to minimize leakage and backflow. Because of this, the thickness of the PDMS was vital in assisting the securement of the Luer lock adapter since threaded end has a large surface area to hold onto without blocking the opening of the channel. The tubing connected from the Luer lock adapter to another adapter where the syringe is attached.

### Final Prototype

In figure 14, the channel is attached at its inlet port to the tubing and a series of Luer lock adapters. The adapters allow the syringe, tubing and channel to be managed separately when need be. Each is able to be disconnected for replacement of individual parts which is necessary if the device is to be used in practice.

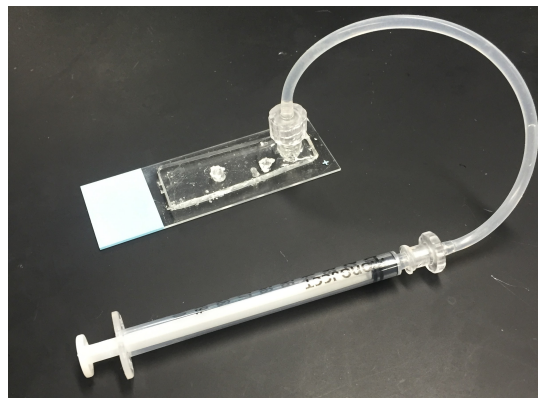


Figure 14: PDMS channel with syringe connected through tubing and Luer locking adapters

The overall device size and portability requirements led to the fabrication of the box encompassed device as seen in figure 15. All components were combined into an electrical box in order to present the device in a clean, easy to use manner. This included the circuitry, the NI-DAQ which was used for data acquisition, and the channel. A syringe connects to an inlet port at the top of the box which attaches to the channel through tubing. The box allows the NI-DAQ to be connected to a computer with the appropriate software for collecting data.



Figure 15: Final box enclosure. Syringe attaches at top of box and sends a diluted blood sample through tubing and the microchannel

## Testing

The cell filtering design as well as the active pumping syringe design were both evaluated. To determine the effectiveness of the proposed filtration method, cell counts were measured before and after being filtered. Experiments were set up to evaluate the ability of the microchannel to count cells as they cross the aperture after being manually passed through by syringe.

## Filtration

To obtain an accurate RBC count using the channel, a filter was devised to separate WBCs from RBCs. The 10  $\mu\text{m}$  pore size was chosen to exclude WBCs, which have a diameter of 12-15  $\mu\text{m}$ , from RBCs, which are 6-8  $\mu\text{m}$  wide. [13] The pore size, which is intermediate between that of red and white blood cells, allows macrocytic RBCs up to 10  $\mu\text{m}$  in diameter to pass through the filter. The filtration device was fabricated by placing a circular polyester membrane filter with a diameter of 47 mm and a pore size of 10  $\mu\text{m}$  onto the open end of a 50 mL centrifuge tube. A round cut was made on the plastic cap to allow filtered blood to flow directly into a tube, and the cap was screwed on, securing and stretching out the polyester membrane (Figure 16). A solution of blood in PBS diluted 1:5000 (1  $\mu\text{L}$  of blood in 5 mL PBS) was poured into the tube containing the filter, and cell counts of the unfiltered and filtered solutions were obtained via use of a hemocytometer.



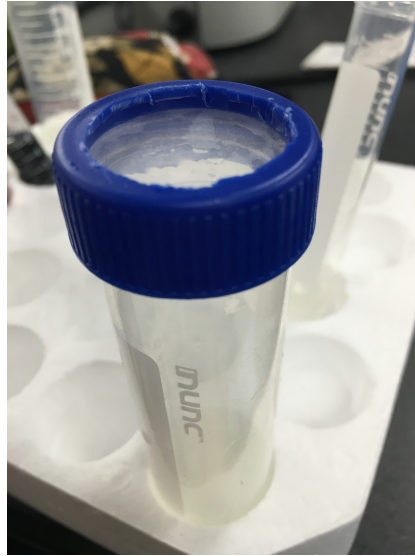


Figure 16: The fabricated filtering method used to gather filtration data

It is expected that cell count before and after filtering will give an indication of the amount of cells lost through the overall filtering process. For the purpose of this device, ideally only large white blood cells will be removed throughout the process. White blood cells make up only 1% of whole blood, so in order to support the hypothesis that only a small portion of cells (white blood cells) are being filtered, the cell counts before and after filtering need to show no significant difference. [14]

### **Pumping and Data Acquisition**

Trials were performed using both active and passive pumping techniques to send a diluted blood sample through the device. Electrodes were fitted each time to measure changes in voltage as cells passed through the aperture.

### **Active Syringe Pumping**

The channel was fitted with the Luer lock adapters and tubing to test how well the channel could measure changes in voltage as cells passed through its aperture. The channel was placed under a microscope so the movement of cells could be viewed and matched with collected data. A voltage divider circuit was created with the channel representing one resistor of that circuit. (Figure FROM PAST WORK) As cells pass through the aperture, the resistance of the channel is expected to increase, therefore increasing the output voltage which is measured through an NI-DAQ data acquisition device. The overall set-up can be seen in figure 17.

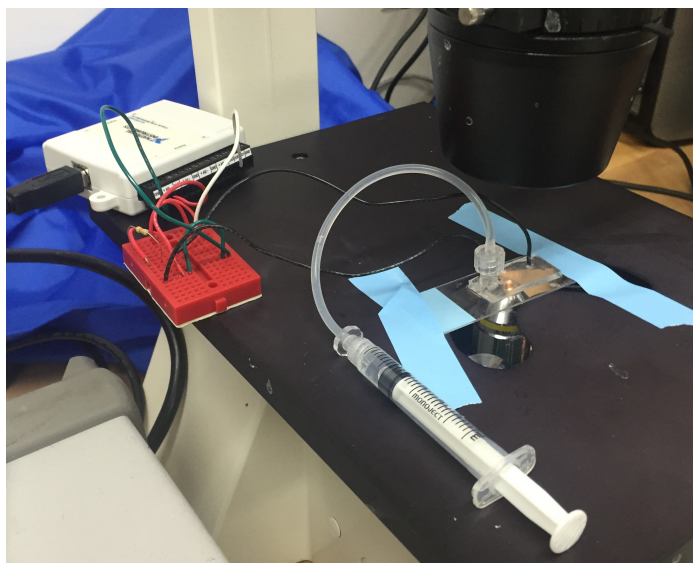


Figure 17: The experimental set-up used for active filtering. The microchannel is placed under the microscope while electrodes from a voltage divider are attached at ports in the channel. An NI-DAQ measures the output voltage.

A porcine blood sample was diluted 1: 5000 in PBS. The syringe was filled with the diluted blood solution and attached to the tubing and channel. Labview software was run as the blood sample was manually passed through the micro-channel. Video was also recorded as cells flowed through the aperture.

### **Passive Pumping**

An identical set-up as above was performed without the syringe and tubing attachment. A fresh PDMS channel was plasma coated to a glass slide and placed under the microscope. Electrodes were placed in the ports of the channel as they were with active pumping trials and data was recorded as cells passed through the aperture.

With passive pumping, a drop of diluted blood solution was placed on the inlet port of the channel and cells flowed freely down the channel towards the outlet port.

## **Results**

### **Filtration**

Statistical analysis revealed that there was a significant difference ( $p$  value  $<0.0001$ ) between cell concentration in the unfiltered and filtered samples (see Table 1). The large disparity between cell counts indicates that the filter was not working as expected since it was hypothesized that filtration would only remove a small portion of cells. This analysis leads to the belief that red blood cells are also being removed in the filtering process, which would affect the accuracy of the overall device. From this experimental data and analysis, it was determined that a filter would not be necessary since white blood cells only make up a small part of total cell count, which should not drastically affect counting within the

channel. Additionally, by adding a filtration component, design complexity would be increased, which is not ideal for a system used in low resource settings.

**Table 1:** Cell Concentration in Unfiltered vs. Filtered Blood Samples

Blood Sample (1:5000 dilution in PBS)		Cell Concentration in Square (cells/ mm <sup>2</sup> )		Cell Concentration (cells/mL)	P value
		Average per square	Avg. (n=12) +/- St. Dev.		
Unfiltered	Drop 1	64	74 +/- 14	1.5*10 <sup>6</sup>	<0.0001
	Drop 2	87			
	Drop 3	72			
Filtered	Drop 1	29	25 +/- 7	5.0*10 <sup>5</sup>	
	Drop 2	21			
	Drop 3	25			

### Pumping Data Collection

Data was collected in LabView and analyzed with a python script written by previous team member Russell Little. The analysis software filters and fits the data to a curve leveling voltage measurements and removing instrumental drift.

### Active

The motion of manually pumping a blood solution through the channel showed to give great fluctuations in voltage. (Figure 18) Ideally there would be a constant base voltage which represents the resistance of the channel with no cells present and as cells pass through the aperture, distinct spikes in voltage are recorded. Over many trials, none of these expected spikes were able to be distinguished; even microscoping video of the channel verified that cells were passing through the aperture. The constant fluctuating change in voltage over time is believed to be caused by the varying pressure applied to the syringe. As different pressures are applied to the channel, changes in resistance and noise cause the data to fluctuate. There are many more contributing factors that prevented the recording of voltage changes and are discussed further in the sources of error.

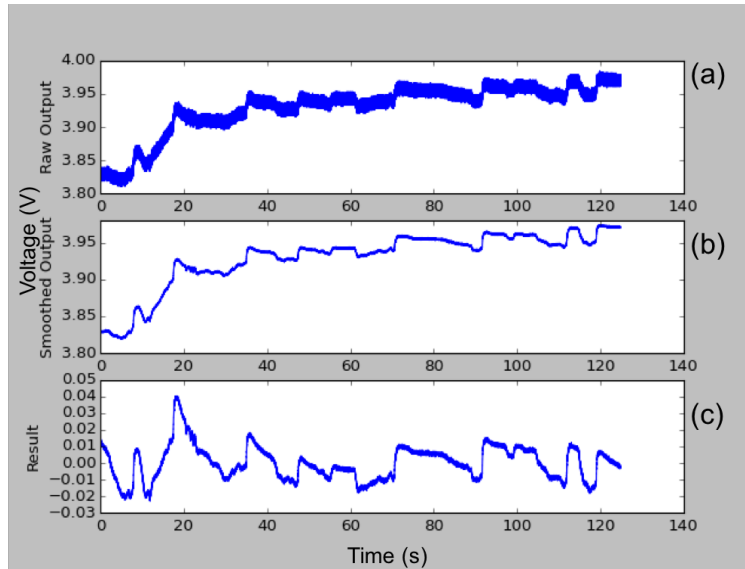


Figure 18: Voltage over time as blood cells were actively pumped through micro-channel. (a) The raw output data (b) Filtered data (c) Curve fit data to level voltage

### Passive

Utilizing the passive pumping technique resulted in a few trials where distinct dips in voltage were recorded. (Figure 19) With the microscope it was verified that these dips did correlate with cells passing through the aperture but not all cells caused a corresponding change in voltage. Over time results became more inconclusive with minimal distinct changes in voltage. Many trials were performed but due to the lack of voltage changes, results were inconclusive. Potential explanations for the lack in substantial data is discussed further in sources of error and future work.

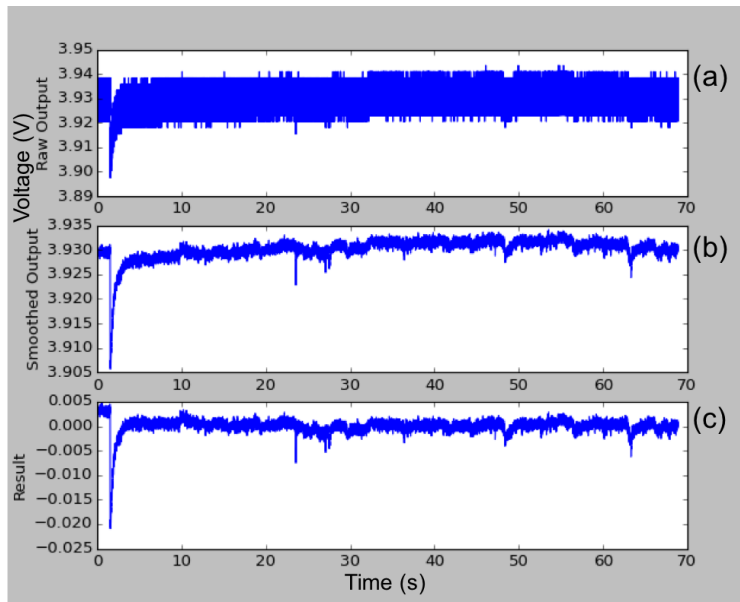


Figure 19: Voltage over time as blood cells were passively pumped through micro-channel (a) The raw output data (b) Filtered data (c) Curve fit data to level voltage

## Discussion

For the filtering testing results, it is apparent that there is a significant difference in cell number before and after filtering. This is concerning because it is obvious that our filter was removing red blood cells and white blood cells from the solution, when the original idea of the filter was to remove only the white blood cells. Because white blood cells only make up approximately 1 percent of the blood, there should not be a significant difference in the cell counts, but the white blood cells would still have been removed from the solution. [14] Though we deemed the filter unnecessary for testing, there is still a potential for white blood cells to enter the device. Due to their size, they could block the channel and prevent an accurate reading. Therefore, further research and brainstorming will need to be done for determining an optimal method for removing the white blood cells prior to the blood entering the channel.

For the pumping results, it was found that the syringe did not deliver a consistent flow rate, therefore causing erratic fluctuations in the data compared to the passive pumping technique. It is thought that an automated pumping system could solve this problem to deliver a consistent flow rate. In the “Lab on a Chip” paper found, the team of researchers from Toronto uses a pneumatic pressure device to consistently move the cells through the channels. [8] This technique may be something to consider, and is discussed further in future work.

## Ethics

The assessment of this device relies on the availability of appropriate blood samples. This project takes a small blood sample and runs it through a microchannel to test for the presence of anemia in the patient and what type of anemia it is. It is important that all blood samples used in the testing of this device come from consenting sources and all approval needed prior to using human samples has been made.

The porcine blood that was used for the testing of this project was humanely gathered and donated to be used by a laboratory on campus.

## Necessary Changes

The results gathered above demonstrate the need to redesign some device components. The fluctuating voltage observed through manually pumping solution through the channel shows the need for an automated pumping mechanism. It’s predicted that applying a constant pressure to the syringe would eliminate the majority of these fluctuations and yield baseline results similar to those of passive pumping.

Due to budget constraints, the channel design from the previous team was used for testing. The previous team designed the channel to work well while passive pumping but some minor tweaks to the channel could make it more reliable with active pumping. A suggested channel design is shown in figure 20. There was a tendency for solution to travel down the shortest and nearest path so the new design incorporates two equal length outlet ports (an electrode will be placed in either). This will eliminate the favorability of one outlet over the other and allow comparable flow to each. In addition to a new

channel, building cups around all channel outlets, allowing solution to pool, would prevent the overflow of solution.



Figure 20: A potential future channel design where the outlet ports are of equal length therefore flow does not favor one over the other.

## Sources of Error

There are some known variables that may have affected the results of this semester's work. In order to build the PDMS channel, the master mold from the previous team was used. Over time, the photoresist of the master started to wear and flake off. This made the fabrication of each channel more difficult and altered the overall dimensions and shape which may have had an effect on the testing results.

A variety of ion solutions were used to dilute blood samples including 1X Phosphate Buffer Solution (PBS), an isotonic NaCl solution and a hypertonic NaCl solution. The conductivity of each solution may have had a more significant effect on the results than expected. This was a trade off between using a more conductive solution that would affect cells over time and a less conductive solution that would have minimal effects on the cells.

Channel blocking was a recurring concern with the channel being that the aperture was only 15 um in size. Any sort of debris or larger sized cell could clog the aperture and prevent future particle flow. The clog would in turn affect the resistance of the channel. With passive pumping a clog could easily be reversed by lightly tapping the top of the PDMS but active pumping caused more severe effects. The pressure of the syringe lodges the debris further into the aperture making the nearly impossible to release and rendering the channel useless.

A different potential source of error in the results was the age of the porcine blood that was used. The blood was acquired from a local lab on campus and it was stored in a fridge without any other method of preserving it. It is likely that over time the blood could have deteriorated and formed clumps. This would throw off the filtering data as it would be hard to study a consistent sample of the blood.

## Conclusions

A portable, easy to use device is needed to diagnose anemia in developing countries where typical diagnostic methods are not available. This project intended to improve upon a particle counting microfluidic device with the end goal of developing a device that accurately measures MCV of red blood cells. The focus of this project was on two modifications to the previous microfluidic device. The

microfluidic channel was fitted with a syringe pumping mechanism in order to quickly measure red blood cell size and blood filtration was analyzed with the intention of removing white blood cells from a diluted blood solution.

The results from filtering a diluted blood sample showed a significant difference in cell count before and after filtration. This led to a conclusion that filtering the blood sample removes more than just large white blood cells since these make such a small portion of whole blood. The remainder of the design forwent the filtering process. Active and passive pumping of a diluted blood sample through the microchannel was analyzed to find that active pumping causes large fluctuations in channel resistance reducing the ability to distinguish small voltage changes caused by cells. Passive pumping showed evidence of voltage changes as cells pass through the channel aperture but trials over time showed inconsistent results which prevented any significant conclusions.

## Future work

There is a multitude of work that can be done on this project, the most pressing being to redesign the device to function as was originally intended. This means taking a blood sample from a patient, directly inserting it into the device and getting a usable reading that would diagnose anemia as well as the type of anemia present, within a minute, that would be accurate to about 95 percent. At this current time, it was shown that syringe pumping by hand is not practical because it creates unsteady flow which causes a varying signal that is not readable. Some sort of automation would need to be introduced into the device to create a smooth and continuous flow, however, this is difficult considering the cost and size constraint of the project.

More work would need to be done in order to improve the functionality of the channels, this includes making the flow more predictable through the channel. Another key improvement that needs to be made is the overall production of the microchannels, currently it takes over an hour and a half to create a single channel. This process needs to be made quicker and more efficient to help make testing new designs faster, as well as making it easier to fabricate larger quantities at a time. Along with this, a better method to attach the tubing to the channels is needed. Currently, the seal between the channel and the tubing leaks and this leakage could harm the circuitry over time.

Using the microchannels, a curve would be made that relates voltages to different sized microparticles. Microparticles of various known sizes would be used, and because they are spherical, their volume could be simply calculated using their diameters. This would then be used to correlate volumes to voltages. Using this curve, it would be possible to deduce the size of the cells that pass through the channel by relating the voltage to the related volume.

Some other work that could be done in the future includes cleaning up the prototype to be a presentable, marketable product, also making it more compact. This would also include switching from the NI-DAQ unit to a microcontroller to simplify the circuitry as well as removing the need for a laptop or computer. At this point a screen would be attached to the device to give the readouts of the results of the test. It would also be beneficial to the end consumer to make the various parts of the device easier to install and replace, including the necessary microchannels and the various wiring and electrodes associated with the circuitry.

Eventually it would become necessary to gain IRB approval to test with human blood. This would be a necessary step to be able to prove that the device functions with human blood.

A necessary step that needs to be done in the future is continuing testing of the device and optimizing the design based on the observations and results of these tests to make sure that it functions as intended and that the results of the anemia tests are accurate and reproducible. The device will be updated as necessary until this is achieved.

Another necessary step in the future will be to test the function of the device with an average person who has had no experience with microfluidic channels or devices similar to this one. This test will help make it clear whether or not the device needs to be simplified in order to be used by a general technician at a health clinic in a third world country. In conjunction with this test, a general manual or procedure of operation for the device will need to be written up. This manual will be given at the time of the above test, and will be revised as needed to be understandable and executable without any other prior training. Based on these results, the design of the device will be adjusted as needed until this goal is met



## References

- [1] MayoClinic.org, 'Anemia Symptoms - Mayo Clinic', 2015. [Online]. Available: <http://www.mayoclinic.org/diseasesconditions/anemia/basics/symptoms/con-20026209>.
- [2] V. Planche, S. Georgin-Lavialle. "Etiologies and diagnostic work-up of extreme macrocytosis defined by an erythrocyte mean corpuscular volume over 130 fL: A study of 109 patients," *Am J. Hemotol.*, vol. 89, no.6, pp. 665-666, 2014.
- [3] Who.int, 'WHO | Worldwide prevalence on anemia 1993-2005', 2015. [Online]. Available: [http://www.who.int/vmnis/anaemia/prevalence/summary/anaemia\\_status\\_summary/en/](http://www.who.int/vmnis/anaemia/prevalence/summary/anaemia_status_summary/en/).
- [4] Globalbrigades.org, 'Global Brigades | Global Brigades', 2015. [Online]. Available: <https://www.globalbrigades.org/>.
- [5] Chiang, M., Choi, U., Enge, J., Little, R. BME 402. "Development of Anemia Detection Device for Low-Resource Setting," 2015.
- [6] W.R. Hogg, W. Coulter; Apparatus and method for measuring a dividing particle size of a particulate system; United States Patent 3557352
- [7] Beckman Coulter. Multisizer™ 3 COULTER COUNTER®, Item No: 6605697. Beckmancoulter.com
- [8] J. Nguyen, Y. Wei, Y. Zheng, C. Wang and Y. Sun, 'On-chip sample preparation for complete blood count from raw blood', *Lab Chip*, vol. 15, no. 6, pp. 1533-1544, 2015.
- [9] Micro-milling. Digital image. Z-microsystems - Moulding of 'Lab-On-A-Chip' Under Clean Room Conditions. Z-micosystems, n.d. 29 Sept. 2015. [Online]. Available: <http://www.medicaldevice-network.com/contractors/nanotechnology/z-microsystems/z-microsystems5.html>.
- [10] Sigma-Aldrich Co. LLC. "Millex® Syringe Filter Units, Disposable, Mixed Esters." *Pore Size 0.22 μm, Diam. 33 mm*. N.p., n.d. [Online]. Available: <http://www.sigmaaldrich.com/catalog/product/sigma/f8148?lang=en&region=US>
- [11] Shields, C., Reyes, C., Lopez, G. "Microfluidic cell sorting: a review of the advances in separation of cells from debulking to rare cell isolation," *Lab on a Chip*, 2015.
- [12] Guckenberger, De Groot, Wan, Beebe, & Young. "Micromilling: A method for ultra-rapid prototyping of plastic microfluidic device," *Lab on a Chip*, 15(11), 2364-2378, 2015.
- [13] Through the Microscope. Wadsworth Center. New York State Department of Health. <http://www.wadsworth.org/chemheme/heme/microscope/rbc.htm>
- [14] Blood Basics. American Society of Hematology. <http://www.hematology.org/Patients/Basics/>

## Product Design Specifications

Client: Dr. Philip A. Bain

Advisor: Professor Thomas Yen

### Team:

Carly Hildebrandt	childebrandt@wisc.edu - Team Leader
Michal Adamski	adamski2@wisc.edu - BWIG
Katherine Barlow	kbarlow@wisc.edu - Communicator
Lazura Krasteva	lkrasteva@wisc.edu - BSAC
Jeffrey Wu	jwu69@wisc.edu - BPAG

Last Updated: Decmeber 9, 2015

### Function

Anemia affects many people worldwide and disproportionately affects those in developing countries due to a lack in medical infrastructure to properly diagnose the blood disorder. A portable, easy to use, and cost-effective device is needed to diagnose the condition in these countries at the time of initial medical care. Anemia can be diagnosed by evaluating red blood cell size using the mean corpuscular volume (MCV). The goal is to fabricate a microfluidic device that effectively measures the MCV of red blood cells to determine if a patient has normocytic, macrocytic, or microcytic anemia with results comparable to current cell counting techniques.

### Client requirements

- Device should provide an accurate diagnosis of anemia and differentiate between microcytic, normocytic, and macrocytic anemia
- Device should be low-cost and adaptable to resource-limited environments
- A clinician should be able to use the device easily and reliably after proper training with an intuitive user interface
- The device should be able to diagnose anemia at the point of care

### Design Requirements

#### 1. Physical and Operational Characteristics

a. Performance requirements: The device should be able to diagnose anemia, by identifying patients' Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), and hemoglobin (Hb) levels. Testing and diagnosis time should take less than 30 minutes.

b. Safety: Blood samples must be introduced to the device in a contained environment so that no contamination occurs between device uses and between the user. The device must be used with proper blood collection techniques.

c. Accuracy and Reliability: RBC, MCV, and/or Hb levels should be measured with at least 95% accuracy when compared to standard counting techniques (i.e. Coulter Counter) to allow for diagnosis of anemia. Diagnosis with 95% accuracy should take no longer than 30 minutes.

d. Life in Service: Device hardware should function for at least 5 years, and the blood collection platform will be reusable. Software should be able to be upgraded when necessary.

e. Shelf Life: Device and all attachments should have the ability to be stored for 5 years from the time of manufacture.

f. Operating Environment: The device will be primarily used in developing countries (i.e. countries in Africa, Southeast Asia and South America), so available resources should be taken into consideration. Generally access to most resources is limited so the device should be able to stand alone or run with minimal outside help (i.e. batteries or a small generator).

g. Ergonomics: The device should be easily transportable and usable by clinicians of varying experience and educational backgrounds.

h. Size: The device should be small enough to fit on a benchtop in a clinical setting while maintaining enough portability to transport to areas in need. The circuitry housing should be no larger than 4" x 6" x 10". The microfluidic device should be no larger than 4" x 4".

i. Weight: The device should weigh no more than five pounds (2.3 kg).

j. Materials: Materials should be low-cost and durable. They should also be biocompatible as to not disrupt cell structure as the blood is moving through the device (i.e. PDMS for the microchannels).

k. Aesthetics, Appearance, and Finish: Device should have an output screen to view RBC, MCV, Hb levels, and classification of anemia. Data should be easily interpreted by the user. Circuitry should be hidden from view.

## 2. Production Characteristics

- a. Quantity: One prototype is needed for proof of concept.
- b. Target Product Cost: Product should not cost more than \$200.

## 3. Miscellaneous

- a. Standards and Specifications: To be determined at a later time. Device will be used in developing countries, not covered by the FDA. FDA guidelines will be followed, but approval is not needed.
- b. Customer: Clinicians of varying skill levels in developing countries.

c. Patient-related concerns: Should be able to give patient a diagnosis at point of care.

d. Competition: Existing devices include the following:

- Coulter Counter: A coulter counter measures the MCV, hemoglobin and the red blood cell distribution width. The coulter principle, which uses the direct current impedance method, governs the use of the device.
- HemoGlobe: HemoGlobe is a non-invasive device designed by students from Johns Hopkins University that measures the hemoglobin level of a blood sample. The device measures the parameter and then sends the data to a center for further data analysis.

- Microfluidic card for RBC analysis: Patent - US8034296 B2: The cartridge enables a complete blood count including red blood cell analysis of various parameters. The cartridge channel may also be subject to an electric or magnetic field during operation.

## Data Analysis

Created by Russell Little

```
import utils
from parse_file import parse
from plot import plot
from calc import polyfit, find_peaks, mean, moving_average

def process_raw_data(filename = None,
    maxima = None,
    binsize=20,
    deg=3,
    delta=.004,
    xlabel='Time (s)',
    title = None,
    **kwargs):
    """

    Args:
    =====
    -> maxima T/F detect peaks as maxima or minima
    -> label

    This function is the main entry point.
    given a file it will do the whole processing... thing...
    this is mainly just to make it easier.

    this is very much dependant on the structuer of the datafile!
    If you change acquisition!!! change this!!!!

    """
    # ASSUMPTIONS!!!!!! change these. there may be more subtle assumptions
    # further in.
    inputV = 'Voltage_0' # input signal, to track phase changes
    outputV = 'Voltage_1' # output signal, to track particles
    x = 'X_Value' # time array,

    r = parse(filename=filename, **kwargs) # parse the datafile
    assert(inputV in r) # __ASSUMPTIONS__ we know this to be the input signal
    assert(outputV in r) # __ASSUMPTIONS__ we know this to be the output signal
```

```

# add some useful info

r['input ave'] = [mean(r[inputV])] * len(r[inputV])
r['output ave'] = [mean(r[outputV])] * len(r[outputV])

# =====
''' Smooth the output to remove large peaks resulting from rare noise events '''
r['smoothed output'] = moving_average(r[outputV], n=binsize)
r['bin size'] = binsize

# =====
''' remove instrumental drift. By fitting a polynomial to the
    output signal, then subtracting it. '''
r['polyfit to output'] = polyfit(r[x],r[outputV], d=deg)
r['deg'] = deg

r['output adjusted'] = r['smoothed output'] - r['polyfit to output']

# =====
''' Run the detect peak algorithm to count the peaks in the datafile. '''
mx, mn = find_peaks(r[x], r['output adjusted'], delta=delta)
if maxima:
    r['particles'] = mx
    r['number of particles'] = len(mx)
else:
    r['particles'] = mn
    r['number of particles'] = len(mn)
r['max'] = maxima
r['delta'] = delta

# =====
''' The hard part is done. just plot the results.
    I want to produce one summary graph. that displays the processing pipeline
    and one that is just the results.
'''
plots = [
    ('Raw Output', r[outputV]),
    ('Smoothed Output', r['smoothed output']),
    ('Result', r['output adjusted'])
]
plot(r[x], plots, x_title=xlabel, plot_title=title, measurements=[])

# =====
# and we're done. return the results just for goodness sake.
return r

```

process\_raw\_data()

## Materials

Product	Place Purchased	Price
Polyester 10 um Filter Sample Pack	Steriltech	30.98
Electrical Box	Amazon	7.45
NI-DAQ	BME Department	donated
1,3 mL Syringes	BME Department	donated
Luer Locks and tubing	Dr. Thomas Yen	donated