Microfluidic Cell Sorter

Preliminary Report

Biomedical Engineering Design 400
Department of Biomedical Engineering
University of Wisconsin-Madison
October 7th, 2020

Client:
Skala Lab:
Dr Melissa Skala
Emmanuel Contreras Guzman
Kayvan Simimi

Advisor:
Professor Justin Williams

Team Members:
Josh Zembles
Sara Wagers
Caleb Heerts
Hunter Hefti

Abstract

Traditional cell sorting is conducted by tagging cells with molecules or RNA sequences which can be a difficult and time consuming task. The Skala lab has developed label-free optical signals to sort T cells by activation state. They have shown that the optical excitation, detection, and signal processing can achieve high accuracy to classify quiescent and activated T cells. Through the use of optics techniques, cells can be excited, identified, and sorted based on their activation state. The lab is in need of a device that will allow for this process to be performed within a single device in order to speed up and improve their research techniques. Previous microfluidic designs created by the lab have lacked in their ability to center the cells and slow them down enough to be individually read by the laser system. Current devices on the market are not suitable for the specific application as they do not achieve the focus or speed required. The goal of this project is to produce a microfluidic device that will allow for a controlled, reduced velocity flow of cells through an interrogation window to be analyzed by the lab's experimental set-up.

Table of Contents

Abstract **2**

Table of Contents **3**

Introduction **4**
- Motivation 4
- Existing Devices 4
- Problem Statement 4

Background **4**

Preliminary Designs **5**
- Plinko 5
- Funnel 6
- Snake 7

Preliminary Design Evaluation **8**
- Design Matrix 8
- Final Design 9

Fabrication/Development Process **10**
- Materials 10
- Prototype Fabrication 10
- Testing 11

Conclusions **11**

References **12**

Appendix **13**
- Product Design Specifications 13

I. Introduction

Motivation

Currently, cell sorting is performed using size identification or tagging and labelling the cells with small molecules or RNA sequences. Tagging or sorting by size can be difficult or time consuming to accomplish as well as potentially affect the structure and function of the cell [1]. To combat this, the Skala lab is researching the use of optics to analyze cells label-free which would result in the activation of certain cells, identification, and sorting all being done in a single device. This could help speed up identification, analysis, and research regarding cells as well as aid in the search for cures of diseases such as cancer or immunological deficiencies.

Existing Devices

The current devices on the market often use sheath flow to move and analyze cells. This involves a sheath fluid such as deionized water or PBS being flowed at a high velocity on both sides of a slower moving cell sample solution. The velocity of the sheath fluid will speed up the cell sample as well as focus it in the middle of a sheath as it travels [2]. Issues with devices using sheath flow were that the cells would move past the light detector too quickly for the cells to be analyzed. Additionally, once the sheath fluid was slowed down, cells wouldn't maintain their x, y, and z location which made it increasingly difficult to both excite and image the cells. Custom microfluidic prototypes were extremely costly as well ranging anywhere from one to four thousand dollars from sources like FireFlySci [3]. The cost, as well as the uncertainty of the device working, led to the disfavor in purchasing custom microfluidic devices. Generic microfluidic plates themselves don't hold cells in a focused location making them difficult to integrate with the Skala lab optics system.

Problem Statement

The Skala lab has developed label-free optical signals to sort T cells by activation state. These technologies could improve monitoring and quality control of T cell manufacturing. They have shown that the optical excitation, detection, and signal processing can achieve high accuracy to classify quiescent and activated T cells. The next step requires a microfluidic chip to flow the cells at speeds that allow 100's of ms integration time on the detector, and a sorting mechanism that can take input from the electronics to sort separate pools of quiescent and activated T cells. The solution can be commercial or newly designed, and requires a bottom of coverglass to integrate with their system.

II. Background

Cell sorting is the process of separating cells based on expressed characteristics. This can be helpful in a variety of ways like separating different cell types, the expression of a protein, or by their activation state. Fluorescent Lifetime Imaging provides a non-invasive and label-free evaluation of the cellular metabolism of each cell. Fluorescence occurs when an emission lightwave excites molecules within or attached to cells and emits a lower energy wavelength that can be detected by sensors [4]. This method is used in many applications including Flow Cytometry. Flow cytometers use hydrodynamic focusing to center cells within a stream so that they are in the path of the lasers. They use a sheath fluid to surround the stream of cells that can narrow the stream and keep the velocity constant throughout the width of the stream [5]. The sheath fluid is needed because fluid moving at the wall of the channel will be slow and increase as the distance increases from the wall. To prevent the cells from moving to the side and traveling at different speeds, the sheath fluid is used as a buffer between the wall and the stream of cells that can vary in speed without affecting the velocity of the cells.

In order to design a device that will allow cells to flow past the laser and be able to integrate into their system, the Skala Lab has laid out specific parameters that the device needs to hold. As outlined in the Product Design Specifications (see **Appendix A)**, the cells need to pass through the laser one at a time while keeping the diameter of the stream containing cells 20-50 microns. The flow speed of the cells should be around 1 mm/s to allow sufficient integration time for the light sensor to read to emission signals from each cell. In order for the lenses to focus the laser on the cells, the bottom of the device to the stream of cells needs to be about 150 microns in height. The device should be able to fit on a microscope stage and be compatible with their pump system. Also, computational fluid dynamics should be used to simulate various designs to help find the best choice to proceed with and optimize the specifications of the device.

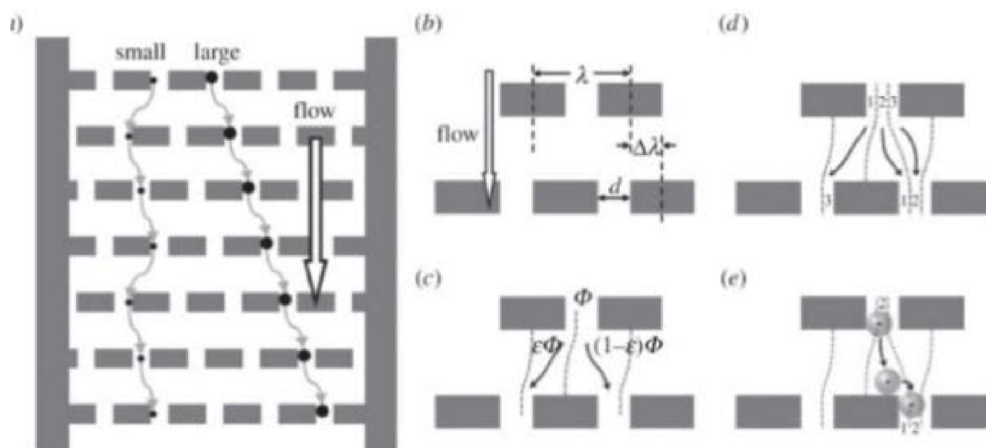
III. Preliminary Designs

Plinko

Plinko is a pricing game which first appeared on The Price is Right in January of 1983. The object of the game is to strategically drop round disks from the top of a peg board in an effort to guide their motion to a specific spot at the bottom of the board. At first glance, this motion appears to be random, but a specific set of mathematical and physical principles does aid in guiding the disk towards an intended location. The parameters that define the motion of the disk can be the angle of trajectory, the shape of the peg, the shape of the object being dropped, or even the weight of the object. But, nonetheless, there is a predictable element that allowed the

creators of the game to mark the price points of certain locations at the bottom of the board based on where the disk was likely to fall [6].

Predictability in the behavioral patterns of objects colliding with pegs on the plinko board provided inspiration for a channel widening technique that uses the same principles. The introduction of obstacles in a channel is not a new technique in microfluidics. Studies have been carried out on the disruption of diffusionally symmetry using ratchets and have shown that objects of varying sizes display predictable motion when flow is asymmetrically disrupted [7].



Sturm *et al.*. *Interface Focus*. **2014**:4(6):20140054. doi:10.1098/rsfs.2014.0054.

Figure 1: A diagram of flow passing through a channel with Brownian ratchets inserted. The basic principle of large particles flowing in an orderly fashion is highlighted.

Using these principles as the basis of the design, the main function of the Plinko concept's introduction to the cellular inlet is to guide the flow of the cells into a central position. Either through the introduction of randomly placed rods into an expanded channel or by directed placement of ratchets aiming cells towards the center of the channel prior to constriction, cellular focusing would be achieved while increasing the volume of PBS flow. Possible implications of this might be the ability for restricted flow or the integration of sheath fluid outlets to slow fluid flow down in tandem with inertial centering.

Funnel

Typical flow cytometry chips are designed with the intent to usher cells towards an objective. The most typical method for driving the sample is through the use of sheath fluid which, in a typical cell, arrives from side channels and converges upon the cellular inlet to carry the sample forward. Sheath fluid designs are frequently used in a two dimensional placement where fluid arrives from one or two directions [8]. While remaining a staple of the experiment, the two dimensional sheath fluid flow has the potential to introduce turbulent flow at the point where convergence occurs.

Alternatives to the two dimensional design commonly include three dimensional alterations. Funnel designs involve the utilization of a cone shaped sheath fluid inlet which allows for complete encapsulation of the incoming cell sample. In these cases, a core diameter is formed in which the cells are centered in all directions within the channel which is guided by laminar flow on all sides in the form of the sheath fluid. For this reason, the Skala Lab has already experimented with variations in a conical design.

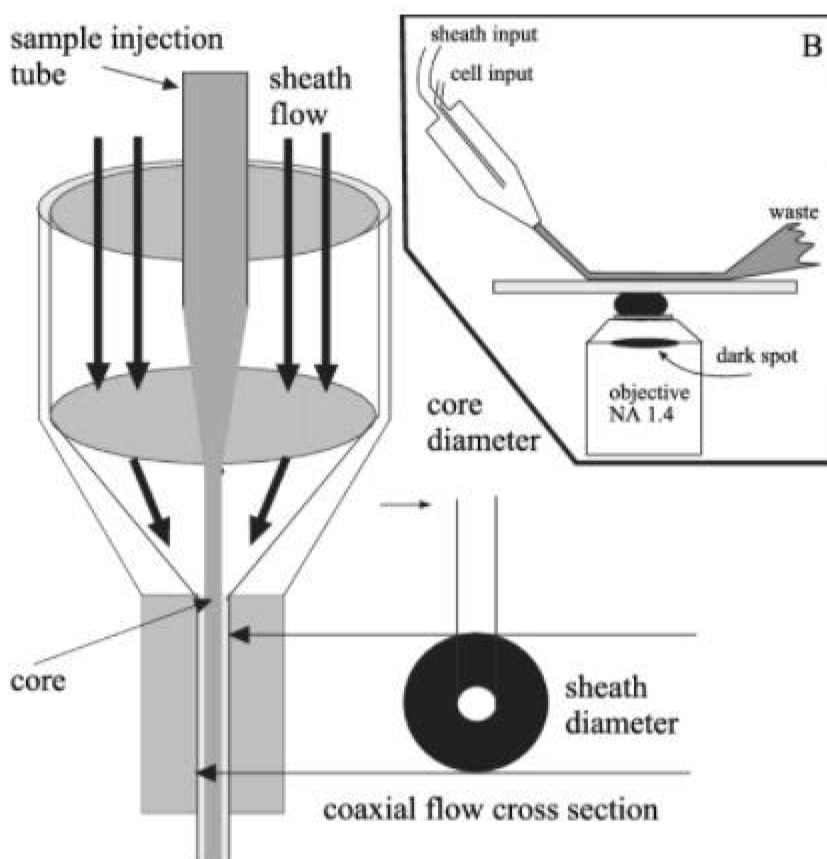


Figure 2: A typical conical shaped funnel design which incorporates angular positioning to bolster fluid speed.

As shown in Figure 2, the funnel design is typically accompanied by an incline in order to induce the fluid and accurately flow on all sides of the cone. While this is beneficial for generating the centering effect, if a kink is involved at the objective transition point, turbulent flow might interfere with the potential benefits of this effect. More simulations on how to adjust for this while retaining the angled pattern would be necessary as well as research into possible improvements as a whole.

Snake

Multiple sources which informed the design process mentioned of a property known as

inertial lift. The concept behind inertial lift is one which is reflected in the diffusion disruption concept that was a main physical assumption underlying the Plinko design. When laminar flow is made to pass through a channel that does not generate uniformly parallel streamlines, flow will seek out a path of least resistance. This displays itself in an outlet streamline which is uniformly centered towards the center of the channel. Such a concept was first introduced in 2007 in which symmetrical designs resulted in two separate cell flows as streamlines were forced towards the sides of the channel via centripetal forces. When an asymmetric design was implemented, a result that bears close resemblance to Figure 3 was produced instead [9].

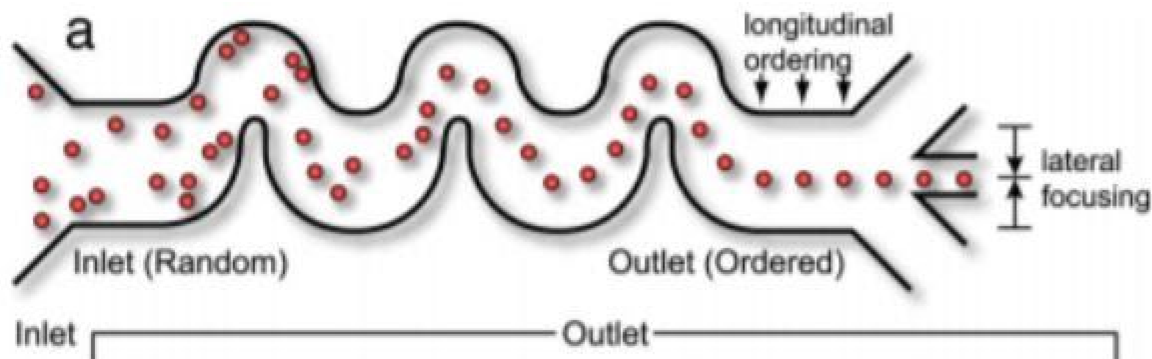


Figure 3: A schematic of a sample prototype for an asymmetrical serpentining channel. The summation of inertial lift forces encourages cells to form a line as they take the path of least resistance towards the outlet.

The snake design was expanded and the concept of inertial ordering was added to a set of potential improvements to cell sorting [2]. Most designs concentrate on cell ordering prior to the addition of sheath fluid. One such design even incorporated an asymmetric squiggle pattern in an effort to create order from the sample directly [10]. But it was hypothesized that adding the sheath fluid prior to entry into the inertial ordering system would allow cells to avoid potential turbulence and order the fluid prior to observation by the laser. Potential additional modifications could then be made to control the speed of flow via the addition of outlet ports that could reduce the volume of flow. Laterally focused cells could be focused at a slower pace and allow maximal ability for analysis with the sorting system.

IV. Preliminary Design Evaluation

Design Matrix

To aid in the consideration of preliminary designs, the team created a design matrix with weighted categories. The most important criteria considered were the Speed Reduction and Positioning. Speed Reduction is a crucial component of the design as it is necessary for the cells to slow down enough to be properly read by the laser. The Plinko design scored highest in this category because the cells will reduce in speed as they bounce around. The Snake design was the

next highest scoring as going through the turns should also reduce the velocity whereas the Funnel design does not consider the need to reduce speed.

The Positioning criteria is as important as Speed Reduction because the cells need to be centered in the x, y, and z axis in order to have consistent readings by the laser. The Snake design scored highest in this category as it was designed specifically to align the cells when they come out of the curves. The other two designs did not score as highly as their alignment mechanisms are not as precisely designed. All three designs may face issues when aligning the cells in the z axis.

Ease of Fabrication is the next highest weighted category because a device that is easier to fabricate can streamline the development process as well as reduce the amount of work that the client will need to do to produce more microfluidics in the future. The Funnel design is the simplest and would be most straightforward to fabricate, followed by the Snake, and finally the Plinko. The client has considered fabrication by an outside vendor which can be expensive, so a simpler design may help to reduce those costs.

The next criteria considered is the Reusability/Sterility. These ideas go hand in hand as it is important that the device can be properly sterilized in order to be used again. The client will sterilize the device by running ethanol and purified water through the device. The Plinko design will likely be the most difficult to properly sterilize as there are many surfaces on which particulates or contaminants could get caught. The other two designs feature smooth channels that should be sterilized easily.

The Manufacturing Cost of the device should be kept to a minimum. This category was weighted lower than others because the cost is not a major concern of the clients, but cost should be reduced wherever possible. All three designs should have comparable manufacturing costs. Finally, the safety of each design was considered, and none of the designs should pose any threat to the user if properly fabricated.

Design Criteria	**Plinko**		**Funnel**		**Snake**	
Speed Reduction (25)	5/5	25	3/5	15	4/5	20
Positioning (25)	3/5	15	3/5	15	4/5	20
Ease of Fabrication (20)	3/5	12	5/5	20	4/5	16
Reusability/Sterility (15)	4/5	12	5/5	15	5/5	15
Manufacturing Cost (10)	5/5	10	5/5	10	5/5	10
Safety (5)	5/5	5	5/5	5	5/5	5
Total (100)	79		80		**86**	

Figure 4: Design Matrix of the three designs discussed above. Criteria are outlined on the left. Each criteria contains a score out of 5 and a weighted score for each design.

Final Design

After conducting an evaluation of the preliminary designs as well as discussing the ideas with the client, the team has decided to not select a single design to move forward with. The clients were intrigued by different aspects of each design and did not feel strongly that one was more suitable than the others. In addition, due to the fabrication limitations that the team will face this semester, the client has expressed a desire for the project to focus more strongly on creating well-developed computational models of the designs. As the focus of the project will be computational, it will be advantageous to develop models of multiple designs in order to decide which to move forward with fabricating.

V. Fabrication/Development Process

Materials

In creating a microfluidic device, the optical properties of the device need to be considered. Due to the experimental set-up, the materials selected need to allow for a laser of up to 450 nanometer light to shine through without causing too much light scattering for the epi-fluorescence microscope. Materials suitable to provide the desired optical properties include glass and quartz, so the bottom face of the device should be made from one of these materials or one with similar properties.

While it is crucial that the laser can shine through the bottom surface of the device, the other surfaces can be made of other materials. A popular material for microfluidic applications is PDMS due to its biocompatibility and ease of fabrication [11]. PDMS can be mated to a glass surface through plasma bonding, a technique that the team has the capability to perform in the ECB tissue lab.

It is important to also note that there are limitations associated with PDMS. In the long term, sterility and longevity of the material can be an issue. This may not be a large concern as the clients would be able to fabricate a number of these devices for use in the lab. Additionally, the clients have highlighted that an issue in past designs was turbulence created at the mating site of PDMS and the quartz capillary. Due to these issues with PDMS, the team plans to conduct further research into alternative material choices.

Prototype Fabrication

Because the focus of the team for the beginning of this project will be on developing computational models of the devices, prototype fabrication has become a secondary concern. After the models have been successfully developed, the team plans to fabricate scaled up models of the device in order to more easily conduct flow testing. Through use of resin printers at the

MakerSpace, highly detailed, scaled-up versions of the devices can be 3D printed. Members of the team have prior experience with using these printers which should lead to success in fabrication.

Once a final device has been designed and sufficiently modeled, there are a number of options for fabrication. First, the client has expressed interest in using an outside vendor for fabrication of the device. This would likely be a simple approach for the team and may provide the client with higher-quality results than could be produced otherwise. For earlier prototypes of the device, glass pieces can be fabricated by the UW Glass Shop and PDMS or other material components can be fabricated in the ECB tissue lab.

Testing

Testing for this project will advance with the development and the optimization of the device. Before fabricating the device we will conduct fluid modeling on Solidworks and compare different modeling constraints. Specific dimensions and fluid speeds will be optimized to find the best design. Once the most promising design is selected, it will be fabricated and tested with the clients laser configuration with fluorescent beads that simulate cells.

The client has offered to test the device with their set up to see if the device holds to their specifications. To simulate cells, fluorescently labeled beads will be used to flow through the device. The first test would include using a brightfield setting on their microscope to see if the beads are flowing in the center of the channel, if they are going through one-by-one and make sure they are flowing at the right speed. The next test would include using the laser to confirm that the beads are flowing through the window of integration and are within the focused laser.

VI. Conclusions

Ultimately, the team's goal is to design a microfluidic device that centers cells within a channel in a single file line so that each cell can be hit by the laser. The Skala lab would like this device so that they can further advance their T-cell research. The next steps in developing this device will involve the team creating 3D models and running simulations to evaluate the best option to fabricate and testing the device to see how it compares to the simulations.

There is still quite a bit of research and consultation required going forward on this project when it comes to finalizing design details. The team has experience modeling in SolidWorks and will utilize the fluid modeling simulation module to optimize and compare designs. In addition, the team is continuing to work with the Skala lab to figure out testing procedures once a device is fabricated. Until then, the team will be working on steps towards finishing the designs and modeling to progress further into the design process.

VIII. References

[1] J. Haron, “Flow Cytometry and Cell Sorting: A Practical Guide,” *Mater. Methods*, Sep. 2020, Accessed: Oct. 07, 2020. [Online]. Available: /method/Flow-Cytometry-and-Cell-Sorting-A-Practical-Guide.html.

[2] P. P. A. Suthanthiraraj and S. W. Graves, “Fluidics,” *Curr. Protoc. Cytom. Editor. Board J Paul Robinson Manag. Ed. Al*, vol. 0 1, p. Unit-1.2, Jul. 2013, doi: 10.1002/0471142956.cy0102s65.

[3] “Custom Quartz Flow Cell Manufacturing,” *FireflySci Cuvette Shop*. https://www.fireflysci.com/custom-quartz-flow-cell-manufacturing (accessed Oct. 07, 2020).

[4] J. W. Lichtman and J.-A. Conchello, “Fluorescence microscopy,” *Nat. Methods*, vol. 2, no. 12, Art. no. 12, Dec. 2005, doi: 10.1038/nmeth817.

[5] J. P. Golden, G. A. Justin, M. Nasir, and F. S. Ligler, “Hydrodynamic focusing – a versatile tool,” *Anal. Bioanal. Chem.*, vol. 402, no. 1, pp. 325–335, Jan. 2012, doi: 10.1007/s00216-011-5415-3.

[6] “Plinko,” *The Price Is Right Wiki*. https://priceisright.fandom.com/wiki/Plinko (accessed Oct. 07, 2020).

[7] J. C. Sturm, E. C. Cox, B. Comella, and R. H. Austin, “Ratchets in hydrodynamic flow: more than waterwheels,” *Interface Focus*, vol. 4, no. 6, p. 20140054, Dec. 2014, doi: 10.1098/rsfs.2014.0054.

[8] A. Cossarizza *et al.*, “Guidelines for the use of flow cytometry and cell sorting in immunological studies,” *Eur. J. Immunol.*, vol. 47, no. 10, pp. 1584–1797, 2017, doi: 10.1002/eji.201646632.

[9] D. D. Carlo, D. Irimia, R. G. Tompkins, and M. Toner, “Continuous inertial focusing, ordering, and separation of particles in microchannels,” *Proc. Natl. Acad. Sci.*, vol. 104, no. 48, pp. 18892–18897, Nov. 2007, doi: 10.1073/pnas.0704958104.

[10] A. A. Nawaz *et al.*, “Intelligent image-based deformation-assisted cell sorting with molecular specificity,” *Nat. Methods*, vol. 17, no. 6, Art. no. 6, Jun. 2020, doi: 10.1038/s41592-020-0831-y.

[11] “PDMS: a review on polydimethylsiloxane in microfluidics,” *Elveflow*. https://www.elveflow.com/microfluidic-reviews/general-microfluidics/the-polydimethylsiloxane-pdms-and-microfluidics/ (accessed Oct. 07, 2020).

X. Appendix

A. Product Design Specifications

Microfluidic Cell Sorter
Project Design Specifications

Team:

Josh Zembles
Sara Wagers
Caleb Heerts
Hunter Hefti

Date:

September 18th, 2020

Function: The Skala lab has developed label-free optical signals to sort T-cells by activation state. The next step in their research requires a microfluidic chip to flow the cells at speeds that allow 100's of ms integration time on the detector. The device can be commercial or newly designed, and requires a number of specifications in order to integrate with their system. The function of the device should create single-file cell flow through the interrogation window with a stable core diameter of 20 um to 50 um while ensuring that stability is first maintained in the z direction. Cells should flow through the microfluidic device along with a PBS sheath fluid at a flow speed of 1 mm/s and up to 10x faster.

Client Requirements: There are a number of specifications that need to be considered in order to ensure that our design is fully compatible with the equipment used by the Skala Lab:

- The device should be able to fit within their microscope's stage insert
- The bottom of the flow cell must have 150 micron glass thickness while accommodating the 1 inch wide objective lens at a working distance of 0.2mm.
- This device should be created with a budget of $2000 in mind, aiming to save money as compared to custom microfluidics and the cost of flow cytometers.

Design Requirements:

1. Performance Requirements: The device must be able to maintain sufficient pressure to flow the cells and media through the channel at a consistently low flow rate. Ideally, the device will be effectively integrated with the pump system that the Skala Lab has already set up. The microfluidic chip should maintain consistent performance over time as it is intended to be a reusable device.

2. Safety: There are limited safety concerns regarding the development of this device. The device should pose no threat to the user if used correctly as all cells and fluids should be contained within the channel. When operating the device or handling any associated cell cultures, typical safety protocols should be adhered to.

3. Accuracy and Reliability: This device must operate accurately to ensure that cells are within the interrogation window for a suitable amount of time. The channel must reliably create a single-cell flow of 1 mm/s and must also limit the variance in z-direction of the cells as they flow through. An accurate device will ensure that experimental data is useful within and between experiments.

4. Life in Service: The life of a flow cell is vague as the potential for reuse is essential to its design. Laboratory glassware can be used indefinitely as long as proper maintenance is applied to keep the material clean. The design will likely be made from glass or quartz as listed below. These items are not particularly prone to a quick expiration. Prototype designs should have a lifespan of at least a few weeks in order for testing to be completed while the final design should have a lifespan that exceeds 10 years if necessary and if proper maintenance is applied.

5. Shelf-Life: In conjunction with the life in service, the flow cytometer cell should be designed in such a way that parts do not degrade while in use. As such, while not in use, the cell should be able to withstand an extended period of resignation in storage that surpasses the lifespan of a cell that is in continuous circulation. This assumes that, prior to storage, proper sterilization techniques using ethanol are employed to prevent mineral build-ups or the proliferation of any residual cells.

6. Operating Environment: Elements of the cell will be exposed to a pulsed laser and should be able to withstand such exposures. Placement under a microscope or under other varieties of imaging equipment may also be possibilities. Pumps are used to produce the pressure that powers the transport mechanisms responsible for pushing fluid and cells through the cell which should also be accounted for. General lab temperatures and light exposures should also be accounted for if necessary.

7. Ergonomics: The microfluidic cell functions similar to a glass slide used for microscope viewing and can be placed over the laser in a manner that is similar. The human hand is capable of picking up objects that are 1 mm thick with relative ease and only two fingers will be required to pinch together enough strength to pick up and hold the cell. Other

elements such as the pump have already been designed ergonomically in a fashion that allows for the control of pressure and flow to remain in the hands of the user.

8. Size: The objective access window that is meant to carry the Quartz/Glass capillary is roughly 3.5 cm long while the PDMS that currently acts as the inlet and outlet are nestled at either end of the tube. The size of the current cell is about as thick as a 1mm glass slide but can likely be thicker up to ~ 2.5 mm while the whole of the device is 9.6-9.75 x 2 cm in overall size. The current laser is set up to accommodate objects roughly this size so the length of the overall cell should not exceed 10 cm in length and not much more than 2 cm in width.

9. Weight: A reasonable weight to set the design of the cell can be estimated as less than 15 grams. Glass can be reasonably approximated as having a density of 2.5g/cm^3 while quartz has a density of 2.43g/cm^3 and PDMS has a density of 0.965g/cm^3. Using all of these measurements in various combinations using the estimated maximal size of the object above, all calculations yield potential weights that are near or smaller than 15 grams. A device made entirely of PDMS would weigh approximately 5 grams. As such, the weight of the cell is expected to fall near one of these measurements.

10. Materials: The materials used for the design should be biocompatible or bioinert. They should not interact with the cells, cell media, or other solutions such as PBS, DI water, or clean water in order to stop any contamination from occurring. Additionally, the materials used should allow light to pass through uninterrupted for measurements being taken. Materials suggested by the client include either quartz or glass, however for prototypes, PDMS may be used due to its ease of fabrication. The material should be able to be reused and cleaned either with ethanol or an autoclave.

11. Aesthetics: The focus of this design is more on functionality. Being able to align the cells with a certain speed is the main importance meaning aesthetics aren’t a major concern. The materials shouldn’t be sharp when touched and the design as a whole should be relatively small to fit on the stage of the lab’s microscope. Additionally, the material chosen must be transparent to allow light to pass through.

Production Characteristics:

1. Quantity: For the semester, only one product is needed, but if a successful design is found, then more could be produced for analyzing multiple groups of cells at once.

2. Target Product Cost: The client has set a budget of $2000 for the prototype. They are hoping to create a device more cost effective than a custom flow cytometer that can be produced with prices ranging upwards of $4000 [1].

Standards and Consumer Characteristics:

1. Standards and Specifications: There are no federal regulations concerning this device since it is being specifically designed for the clients use. However, the device needs to be sterilized to ensure no contamination.

2. Patient or User-related Concerns: It is incredibly important that this device will maintain sterility and work accurately as it will be used for research experiments. Care should be taken to ensure that cells from different batches are separated and treated as such.

3. Competition: Currently most cell sorting microchips [2] use weight or size as the factor to separate different cells. These kinds of chips will not work since they depend on multiple types of cells while the clients have one type and are either fluorescent or not. The cell sorting techniques that are based on fluorescence are an all-in-one machine. The client only wants the microchip which allows cells to be centered in a stream so their custom laser can be used to identify each cell. Microchips that consist of small channels are available on the market that allow for a stream of cells to flow through a narrow channel under a microscope [3]. However, these cells are not centered within the channel for the laser.

References:

[1] “Custom Quartz Flow Cell Manufacturing,” *FireflySci Cuvette Shop*. [Online]. Available: https://www.fireflysci.com/custom-quartz-flow-cell-manufacturing. [Accessed: 17-Sep-2020].

[2] Microfluidic Chip-Based Gentle Cell Sorter, Single Cell & Cluster Dispenser | On-chip Bio. 2020. *On-Chip Sort CONSUMABLES : MICROFLUIDIC CHIP | Microfluidic Chip-Based Gentle Cell Sorter, Single Cell & Cluster Dispenser | On-Chip Bio*. [online] Available at: <https://on-chipbio.com/product-onchip_sort/microfluidic-chip/> [Accessed 17 September 2020].

[3] “Straight Channel Chips - Glass,” *microfluidic ChipShop*. [Online]. Available: https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/glass-chips/straight-channel-chips-glass/. [Accessed: 17-Sep-2020].