# Microfluidic Cell Sorter

Final Report

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# 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. 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. Work this semester included developing flow simulations and CAD drawings for two potential designs. Initial simulation testing has shown promising results with regard to the ability to center the cells in the microfluidic channel, and, upon further refinement a prototype will be fabricated for testing in the lab.

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# 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. Design of a custom microfluidic device to improve their testing system will allow the Skala lab to conduct this research more effectively.

## 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 flowing 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]. The biggest issue with devices using sheath flow is that the cells would move past the light detector too quickly for the cells to be analyzed. Additionally, once the sheath fluid is slowed down, cells don't maintain their x, y, and z location which makes it increasingly difficult to both excite and image the cells. Custom microfluidic prototypes are extremely costly as well, ranging anywhere from one to four thousand dollars from sources such as FireFlySci [3]. The cost, as well as the uncertainty of the device working, led to the Skala lab's disfavor for 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 in their research 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 applications such as separating different cell types by 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 in order to determine the best choice to proceed with and optimize before fabrication of the device.

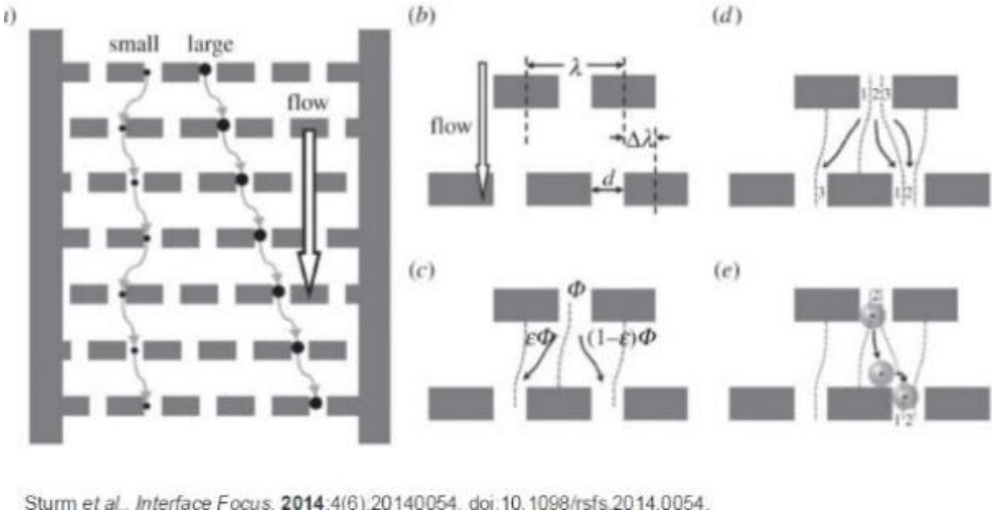
# III. Preliminary Designs

## 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.

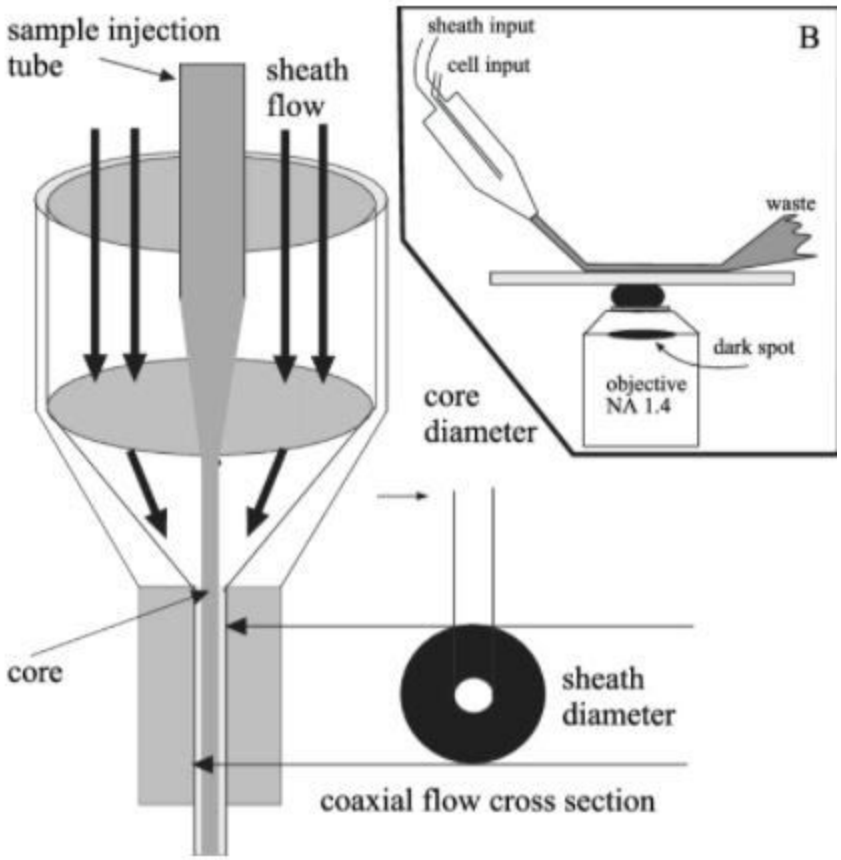



**Figure 2:** A typical conical shaped funnel design which incorporates angular positioning to bolster fluid speed.

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].

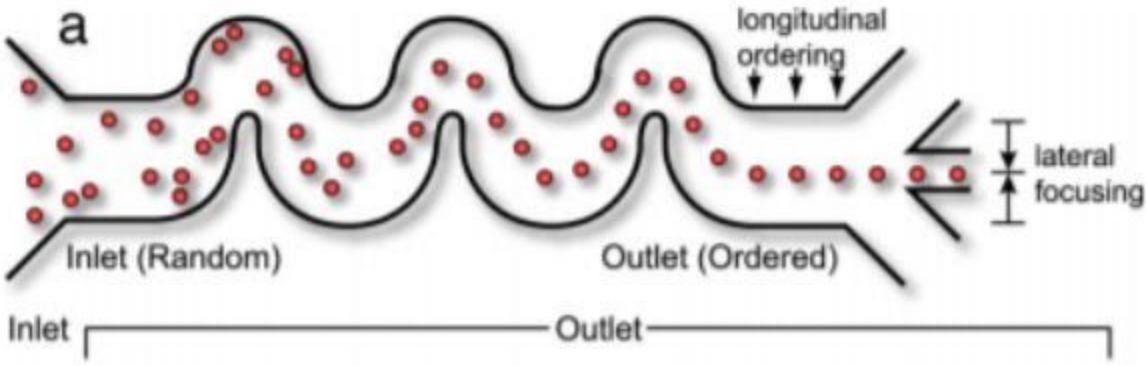



**Figure 3:** A schematic of a sample prototype for an asymmetrical serpentining channel. The summation of inertial lift forces encourages cells to form a line as they take the path of least resistance towards the outlet.

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 axes 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 Designs

After conducting an evaluation of the preliminary designs as well as discussing the ideas with the client, the team has decided to move forward with development of both the Funnel and Snake designs. The clients were intrigued by different aspects of each design and did not feel strongly that one was more suitable than the other upon initial inspection. While the Funnel design has known capability for centering of the cells using sheath fluid, the Snake design has the potential to be able to center the cells at a lower speed without the need for sheath fluid. For these reasons, both designs are being considered and a singular final design will be selected after more thorough testing has been conducted. The clients felt as though the plinko design would be too difficult to manufacture and was lacking in its ability to effectively center the cells, so it was not developed further.

Due to the fabrication limitations of this semester, the client expressed a desire for the project to focus more strongly on creating well-developed computational models of the designs. As the initial focus of the project was computational, there were ample opportunities to work simultaneously on both the Funnel and Snake designs before determining which design will yield better results. SolidWorks models were created for both designs and preliminary fluid flow and particle simulations have been conducted. At the present, both designs are still being developed and considered, and one design will be selected as the final design after further testing has been completed.

# V. Computational Testing

## Funnel Design

The first design the team went forward simulating this semester was the Funnel design. This design was built off of the clients lab's previous design concept. Building this design in SolidWorks allowed the team to simulate and test various parameters with the design. These simulations were intended to ensure the design should be able to center the cells and to learn how changing velocities would impact the behavior of the fluids.

The Funnel design consisted of using a 0.18 mm diameter flat tip needle to introduce the cell solution while a sheath flow surrounded the needle until the end. The sides of the device begin to enclose the fluid into a smaller 0.4 mm diameter channel that carries the cells over the laser for reading. As the fluid moves through the funnel part of the design, the velocity will increase as the diameter decreases. The sheath fluid should confine the cells into the center as the sheath flow is on all sides of the cell flow. The final velocity of the fluid within the channel depends on the speed and the volume of fluid entering the system at the two inlets.

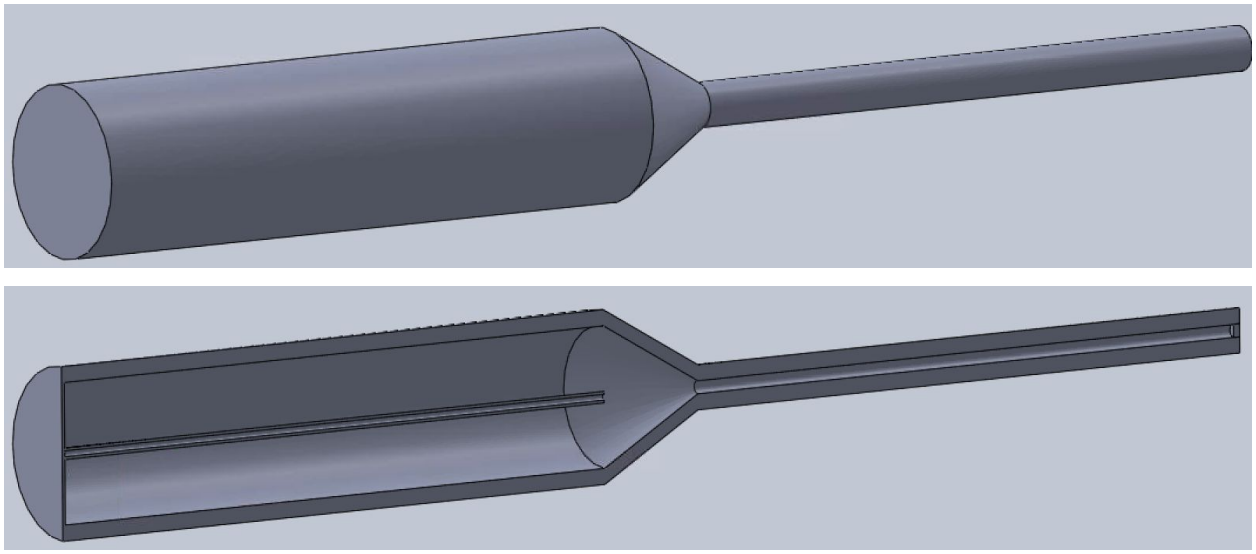


**Figure 5**. An angled view of the Funnel design. A cross sectional view of the design shows the needle that contains the cell solution flow and the sheath flow volume that surrounds the needle.

Flow simulations were conducted once the SolidWorks model was completed. The fluid flow of the needle that contained the cells was examined in order to look at how well the sheath flow was confining the core flow as it traveled into the channel. A particle study was conducted as well to examine the behavior of particles that represented cells would behave in the core flow. Looking at where the particles would end up is a critical component in the simulation as it the goal to center them within the channel. Another simulation that was conducted looked at the

fluid velocity within the channel, an important parameter because the cells need to be traveling between 1-5 mm/s for the laser to efficiently read each individual cell as it passes through. The channel fluid velocity was measured with varying inlet velocity speeds to gain an understanding of the circumstances that would allow for the optimal flow for the laser to read.

## Snake Design

Due to the complicated nature of inertial ordering, multiple iterations of the Snake Design needed to be made in an attempt to test the validity of the concept while making room for various design alterations and potential errors with the simulation software. While tools such as OnShape or COMSOL were considered to help with the modeling and fluid simulation processes, SolidWorks was ultimately decided upon as it would make for good comparisons to the Funnel Design which was already using this software.

Designing the snake would require two variations to be implemented in order to test for the most effective centering technique. One design would focus solely on the creation of a serpentining channel. This channel would undergo a fluid simulation and a subsequent particle simulation before qualitative analysis would reveal if enough of an improvement had been achieved to begin qualitative data collection. The singular channel was designed using a few different methods. Chiefly, a standalone sketch or series of sketches was cobbled together with the intent of generating a sweeped channel that would integrate the internal structure of the design by connecting a series of planes. Alternatively, an outline could be created and then extruded prior to adding in a sweeping cut or a hollowing feature to account for a similar internal structure. These types of standalone designs were useful in determining the success of the inertial ordering method as a standalone system, but they were not spectacular at demonstrating a possible method for manufacture or at showing how a final device might actually look. For this, design methods turned towards extruded cuts within a sheet of material. This type of design was more likely to mimic a device that might be manufactured using molds or engraving techniques.

Aside from testing the inertial ordering by itself, another type of design utilizing similar methods was generated that employed the use of sheath fluid at the outlet of the serpentining channel. Such a design can be viewed in Figure 6. The purpose of this design was to demonstrate an improved efficiency of the inertial ordering method in centering cells. The concept was that, once the Snake had fully accomplished its task, sheath fluid would further center the cells, potentially in another plane, and direct the focus more precisely towards the target. Only a few designs ended up incorporating this.

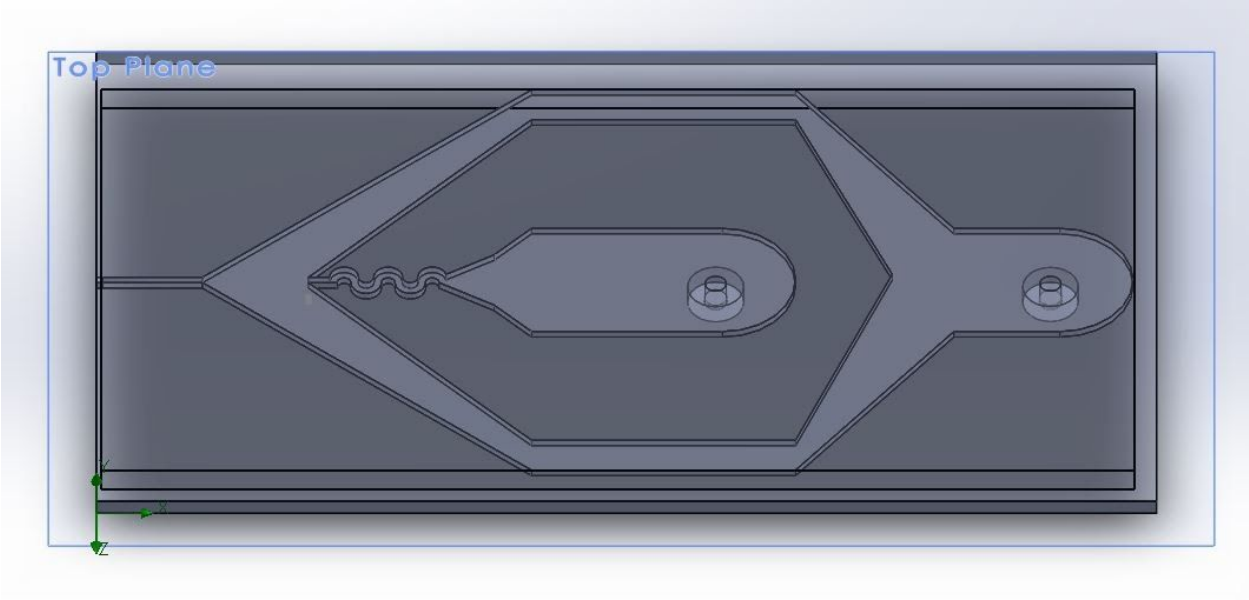



**Figure 6**. An angled view from above of the sheath fluid design. One inlet allows for particles to pass through an inertial ordering system while another allows entry for sheath fluid.

Once a model was completed, a simulation model would be applied. This typically consisted of setting up two or three boundary conditions and running a test for volume flow rate. At the outlet of the device, a static pressure was set; this would ensure that the pressure of the water inside the device would gradually force the fluid to move towards the hypothetical exit. The inlet was set to have a velocity between 0.05m/s and 1m/s depending on the scale of the particular device that was being tested. If sheath fluid was being added, this defaulted to a volume flow rate of $10\,m^3/s$ . Upon completion of a flow simulation, a quantitative analysis of the flow trajectories could be made immediately. This was followed by the insertion of a particle injection, a type of particle simulation in which a quantity of particles is made to enter at a specified entry point and reacts to the flow that surrounds it. Such a simulation was accomplished using 10μm diameter polycarbonate particles; results were observed in a similar fashion.

The client's initial instructions for this design were for the channel to ultimately have a width of approximately 50μm while the cells were estimated to be 10μm in diameter. To analyze the effectiveness of these parameters, a few equations which were used by former researchers in the initial development of this technique had to come into play [9]. The first equation, the Reynolds number, is used to define the much more relevant and important parameter, the Dean number.

$$R_c = \frac{U_m D_h}{\nu}$$

**Equation 1.** Channel Reynolds Number

The Reynolds number is a dimensionless ratio of inertial forces to viscous forces. Inertial forces are typically represented by density, flow speed, and a length scale. In the case of the Snake Design, two separate Reynolds numbers are required for a proper analysis. The Channel Reynolds number is altered by the addition of $U_m$ (the maximum channel velocity), v (the kinematic viscosity of the fluid), and $D_h$ (the diameter of the channel). For an altered form of the equation that uses mean velocity instead, the Channel number is ⅔ of the original Reynolds number.

$$R_p = R_c \frac{a^2}{D_h^2} = \frac{U_m a^2}{\nu D_h}.$$

**Equation 2.** Particle Reynolds Number

The Particle Reynolds number is more a measure of the way the particle behaves within the channel. This can be obtained by multiplying the channel number by another dimensionless constant - the squared ratio of the particle diameter to the width of the channel. Typically, $R_p$ is much less than one, and this implies that the particle will match the speed of the fluid in the channel. This means the $R_p$ has the additional reliance on the diameter of the particle, which makes it a slightly more effective method of quantifying the flow of the particle. Previous research has suggested that the level of focusing increases with the $R_p$. An $R_p$ that is too high will result in damage to the cells, but an $R_p$ that is too low will not result in proper focusing.

$$De = Re(D_h/2r)^{½}$$

**Equation 3**. Dean Number

The Dean number is another dimensionless constant that places reliance on the centripetal forces occurring within a pipe. The major modification is the addition of the r (radius of curvature) term. As before, research suggests that Dean number should be less than 50 to be effective at maintaining flow conditions throughout the channel, and numbers between 10 and 20 are the most efficient for proper cell focusing. Upon further analysis of the research, it was discovered that the ratio of particle diameter to channel width ought to be between 0.1 and 0.5 to conserve this effect. As such, the channel width of 50μm could maintain particles with a diameter between 5 and 25μm before focusing would no longer be effective. The balance between inertial lift and Dean drag force is what maintains the cell localization to a specific point in the channel. Analyzing this number more closely will be vital to further improvements in the snake design.

# VI. Results and Discussion

## Funnel Design

The results of the computational modeling for the Funnel design provided beneficial insight into the feasibility and functionality of the design. The flow simulation modeling revealed information about the flow behavior of the core fluid diameter as it traveled into the channel. The modeling also revealed information on how cells would flow through the system. The parametric study on different inlet velocity speeds also provided information on how different speeds affected the flow.

The simulation of the Funnel design, as seen in Figure 7**,** shows that it was able to reduce the diameter of the core flow as it enters the channel. It was also shown that as the core fluid travels into the channel from the needle, it increases its velocity. As of now, the team was not able to quantify the reduction in the core diameter other than by looking at the streamlines of the flow. Having a better understanding of the reduction of core diameter would give better insight as to where the cells would be traveling down the channel. The cells would most likely stay within the flow of the core fluid, however to provide more evidence of this, particle tracker simulations were used.

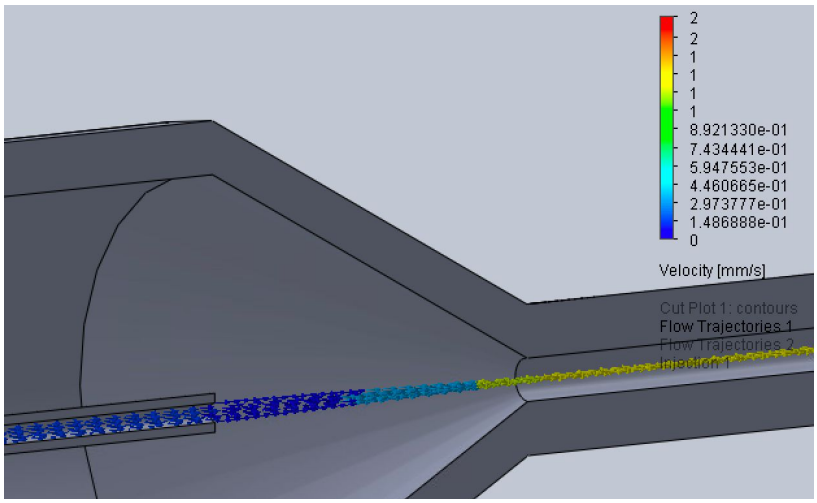



**Figure 7**. A cross sectional view of the Funnel design showing flow of the fluid coming from the needle.

The particle study shows what cells would behave like as they traveled through the device and it shows the cells being confined along with the core flow, as shown in Figure 8. It also further confirms that the cells are being centered within the channel. Another issue the team

had was quantifying where the cells were actually located within the channel. A method to look at where the cells were passing through the cross section of the channel other than looking at the results of the particles traveling down the device has not yet been determined. The team is hoping to be able to find a way to look at how close to the center of the circular channel the cells would be located. However, additional experimental testing would be needed to ensure that the cells are in fact staying within the area of core and if the model was correct.

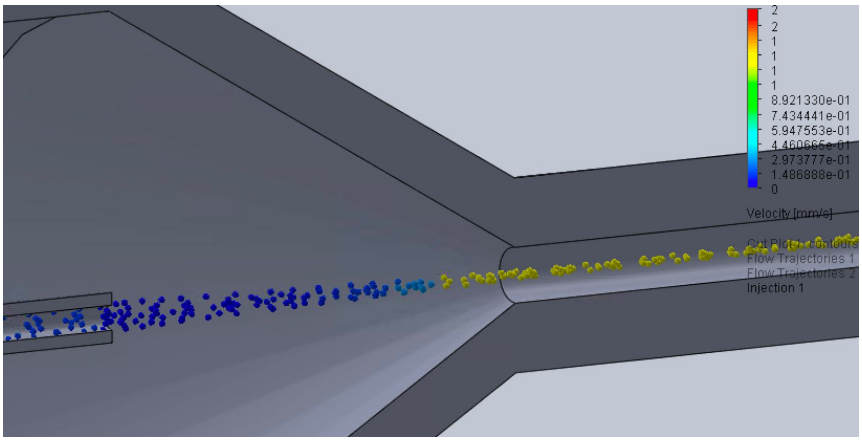



**Figure 8**. A cross sectional view of the Funnel design showing particles that represent cells flowing through the system from the needle.

To achieve the client's goal of having the velocity of the cells around 1-2 mm/s as they pass through the channel, some testing of different inlet velocities was simulated. First, the velocity of both inlets was varied to gain an idea of the effects that it would have, shown in Figure 9. The results revealed that the velocity of the sheath flow had a much greater impact on the flow in the channel while changing the inlet velocity of the cells had little to no impact. Following these results, the cell velocity was fixed while varying the sheath flow velocity to understand how it would need to be adjusted to get a final channel velocity of around 1-2 mm/s. The results conclude that the velocity of the fluid in the channel was dependent on the sheath flow velocity. Another factor that would affect the velocity is the volume of fluid in the sheath flow which could be varied if more control was needed.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Velocity (Cells) [mm/s] | 0.1 | 0.55 | 1 | 0.1 | 0.55 | 1 | 0.1 | 0.55 | 1 |
| Velocity (Sheath Flow) [mm/s] | 0.01 | 0.01 | 0.01 | 0.055 | 0.055 | 0.055 | 0.1 | 0.1 | 0.1 |
| Velocity in the channel [mm/s] | 1 | 1 | 1 | 7 | 7 | 7 | 13 | 13 | 13 |

| | | | | | |
|---|---|---|---|---|---|
| Velocity (Cells) [mm/s] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Velocity (Sheath Flow) [mm/s] | 0.001 | 0.005 | 0.01 | 0.015 | 0.02 |
| Velocity in the channel [mm/s] | 0.148 | 0.656 | 1 | 2 | 3 |

**Figure 9**. Table of final velocities in the channel that corresponds to a variety of velocities of the two inlets.

## Snake Design

For the bulk of the Snake Design results, mostly quantitative analysis was used. This was due to the search for a method to properly analyze the qualitative results of the Funnel Design as well as the various alterations that were made to the Snake in terms of frequency of turns, radius of curvature, speed of flow, and the presence of sheath flow. It was determined that a clear lack of focusing results was not worth further qualitative analysis. The majority of the designs were plagued with technical issues or were not capable of properly focusing the particles in their respective simulations due to design flaws or to improper simulation parameters that did not successfully test the full capabilities of their respective design.

One of the first designs to successfully launch a running simulation was the sheath fluid design. This design incorporated an extra port that fed sheath fluid towards the outlet of the inertial ordering section of the channel. Figure 10 illustrates the resulting particle simulation that was produced. Cells appear to focus early on in the channel and, upon contact with sheath fluid, are focused further within the plane of the fluid and propelled at faster speeds towards the outlet port of the device.

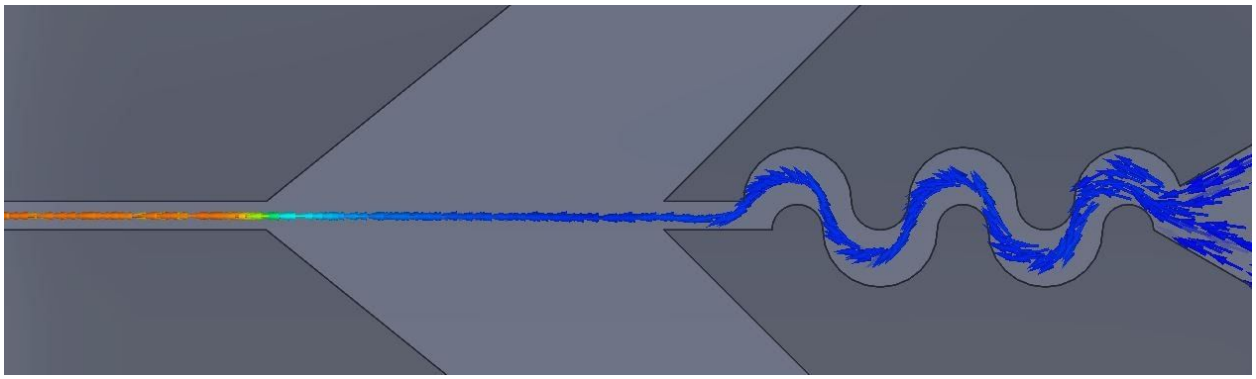


**Figure 10**. A visual representation of the XY plane of the particle simulation with sheath fluid. Increased focus can be observed upon contact with the sheath fluid.

While the success of this design initially seemed to illustrate the feasibility of a shortened inertial ordering combination design, the clients pointed out that the majority of the focus was likely a direct result of the addition of sheath fluid and very little was actually being accomplished by the inertial ordering. Figure 11 shows the result of the sheath fluid being removed from the simulation parameters. Upon reaching a more open channel, the cells will expand to fill more space which reduces the effectiveness of the inertial focusing. While this did not directly disprove the theory behind the Snake, it did demonstrate the need to build a model that could accurately center cells without the need for sheath fluid in order to prove that any effect was actually being demonstrated.

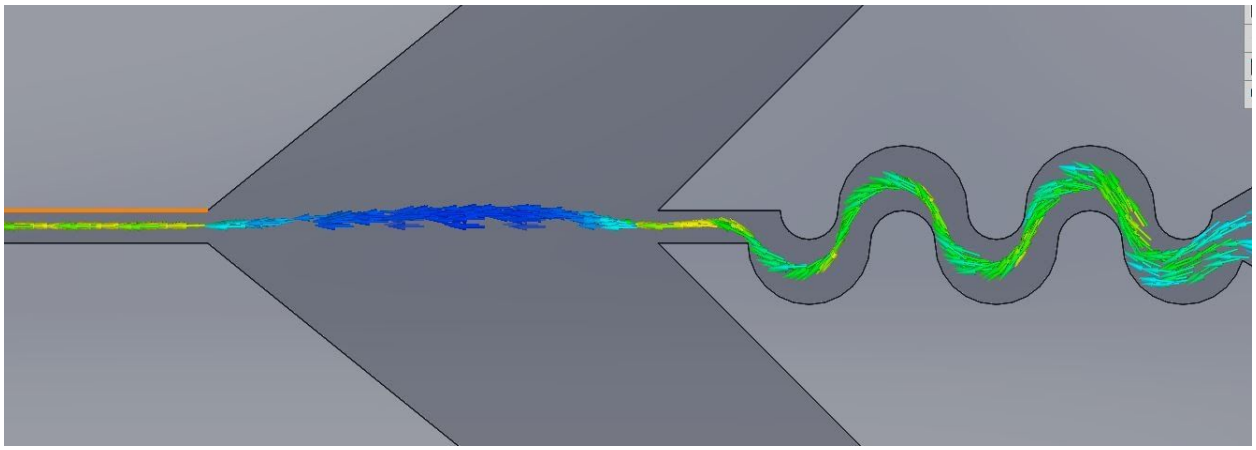


**Figure 11.** A particle simulation using the same model as before but without sheath fluid. Focusing efficiency is dramatically decreased after exiting the inertial ordering mechanism.

A new design was generated to better fit the criteria that the client demanded. Rather than a short repetition of three curves, the new design incorporated fifteen curves to allow for increased focusing. The design was almost identical to the one proposed by Di Carlo [9] with one major exception: the width of the channel curves was almost doubled. This error was not identified until very late in the design process, so tests were run without making a direct alteration. In the future, this error will be rectified so that the small width of curvature equals the width of the normal channel. However, running this simulation at slow speeds of 0.05m/s, the design does demonstrate one interesting physical property. As can be seen in Figure 12, central velocity increases at the outlet of the channel. This would be expected if inertial forces were acting as they were supposed to. Further, this would also imply that the main reason for the design's failure to demonstrate proper results is likely a result of the loss of Dean drag force.

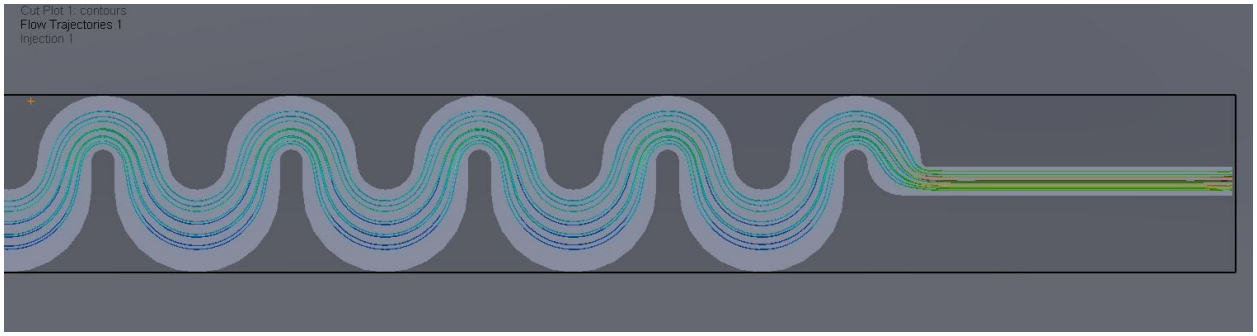



**Figure 12.** Flow trajectories on a separate design. Mirrors the design found in Di Carlo et. al [9] with slight alterations. Initial velocity = 0.05m/s. $R_p = 0.2$

Returning to this design following the final presentations, the team was able to make some minor improvements to demonstrate the effect that $R_p$ has on the focusing. By increasing the initial velocity of the fluid to ~1.5m/s, the exact same design was able to focus cells much more consistently. Figure 13 shows this increased focusing as a result of increasing the velocity.

Thus, the two main sources of error in the latest models seem to stem from the speed of the fluid and the channel width as it relates to the radius of curvature. Overall, results from the Snake Design were less than favorable.

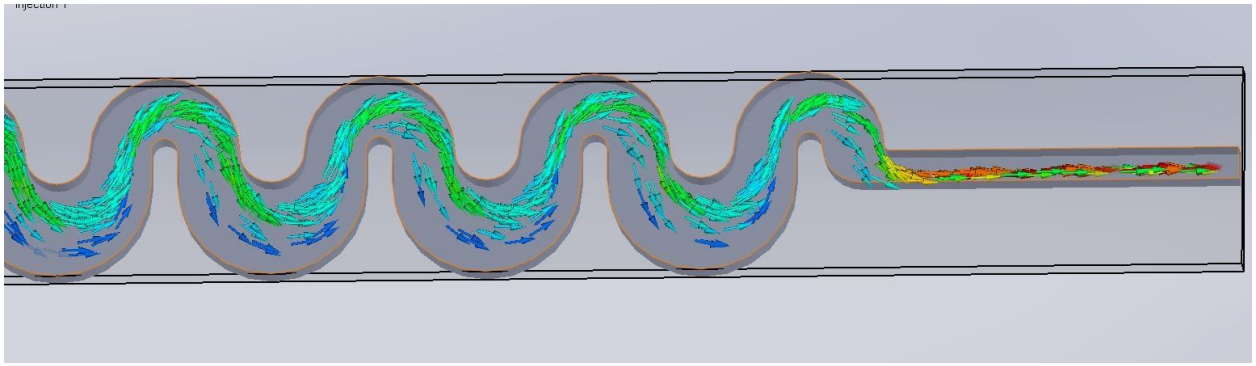


**Figure 13.** Another view of the former design running at a faster speed ($R_p$ = 2.9). Focusing does seem to improve at faster initial speeds.

These designs initially yielded results that seemed unhopeful. However, with correction to the sources of error, it is likely that more improvements to the Snake's ability to focus cells will be observed. Increasing the speed of the flow through the channel was able to increase the effectiveness of the inertial focusing. Reducing the width of the curves from 100µm to 50µm might help account for the speed changes in the Reynolds number calculation. If an $R_p$ of ~3 remains as effective as it appears, then an alteration in the radius of curvature would also ensure that the Dean number is decreased to an appropriate level. The current radius of curvature for the working design is approximately 90µm. By plugging in the appropriate numbers to maintain an $R_p$ of 3, the appropriate radius of curvature can be calculated between 60 and 75µm instead. Making this adjustment will ensure that the drag forces experienced by the fluid flow in the curved channel properly balances out with the inertial lift forces generated by the size of the particles. In the next few weeks, this design will see finalized construction, and the true viability of the Snake Design can be analyzed properly.

# VII. Conclusions

The team had a fairly successful semester with the microfluidic design. Two potential designs were chosen by the client for the team to pursue over the course of the semester, as opposed to the typical one design, which led to a slight time crunch on the team's behalf. Regardless of this, and other setbacks, notably SolidWorks access and functionality, the team was able to create several SolidWorks models, run fluid simulations to look at velocity and pressure profiles, and create particle flow simulations for the designs.

Of the two designs, the funnel design was simpler to model due to the geometry and popularity of flow cytometry. As the model of the design was completed earlier in the semester, more in-depth fluid modeling was performed such as analyzing outlet velocities with varying inlet velocity profiles. On top of this, velocity profiles and particle simulations were run for analysis of fluid and particle behavior through the model. While these models are nice, it doesn't provide comprehensive information on the model. The team hopes to vary other parameters in the model, such as varying the core diameter, as well as test a physical device to get a better look at how well cells are held in the center of the core channel.

Work on the Snake Design was limited to generating various methods for creating the proper model. Once SolidWorks was decided on as the simulation software, most of the modeling process was focused on searching through the literature and determining which of the inertial focusing mechanisms was most effective. While the team did run into technical issues with SolidWorks access early on, they were able to create several different designs over the course of the semester. Eliminating the possibility of adding sheath fluid came quite late in the process, so the early designs that focused on incorporating both elements as centering methods were scrapped. When the Di Carlo paper was found and selected as the most thorough description of a working inertial ordering method to focus on, it was already quite late in the design process, so attempts to mimic these designs were difficult. Plagued with technical errors and limitations, it seemed that all the simulated results of the Snake Design pointed towards failure. However, reliance on the use of Dean number and the drag forces that such a number implied allowed for a few last minute discoveries. The effectiveness of the Snake Design in the future will be dependent on the proper fluid flow mechanics that dimensionless constants like the Reynolds number and Dean number seem to provide. Alterations to the radius of curvature, width of the curved channel, and initial flow velocity will all have to be made if this design is to remain a consideration in the future of this project.

# VIII. Future Work

## 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, PMMA, 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

Prototype fabrication will be a larger concern in the upcoming semester due to all the modeling taking place initially. With the two models discussed above, 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. The team will additionally need to verify that the UW Glass Shop can print at the high resolution required for microfluidic devices, but if unable, the team will discuss external fabrication shops with the client to determine next prototyping steps.

## Testing

Testing for this project will advance with the development and the optimization of the device. Current testing has included fluid modeling on SolidWorks and comparison of different modeling constraints. Specific dimensions and fluid speeds are still being optimized in collaboration with the client 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 team hopes to print a larger scale, lower resolution version of their model using the Makerspace’s microfluidic 3D printer. While testing on this device won’t be in depth, the team hopes to view the flow in the physical design and analyze if the flow becomes turbulent at any points in the design. Of specific concern are the inlets and outlets that connect with the pump the

client's lab uses for pushing cells and sheath fluid through the device. Once the larger scale device is analyzed, the team hopes to print the real micron scale design with help from either the UW Glass Shop or an external microfluidic company.

The client has offered to test the microfluidic 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.

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 hopes to finalize their designs and ideally narrow it down to one final design. On top of this, the team is eager to try and test prototypes whether they're scaled up models, or micron scale microfluidic plates. 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 and evaluating the two designs.

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# XI. Appendix

## A. Product Design Specifications

**Microfluidic Cell Sorter**
Project Design Specifications

**Team:**

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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.

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