I. Appendix I: Project Design Specifications

Project Design Specifications

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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].

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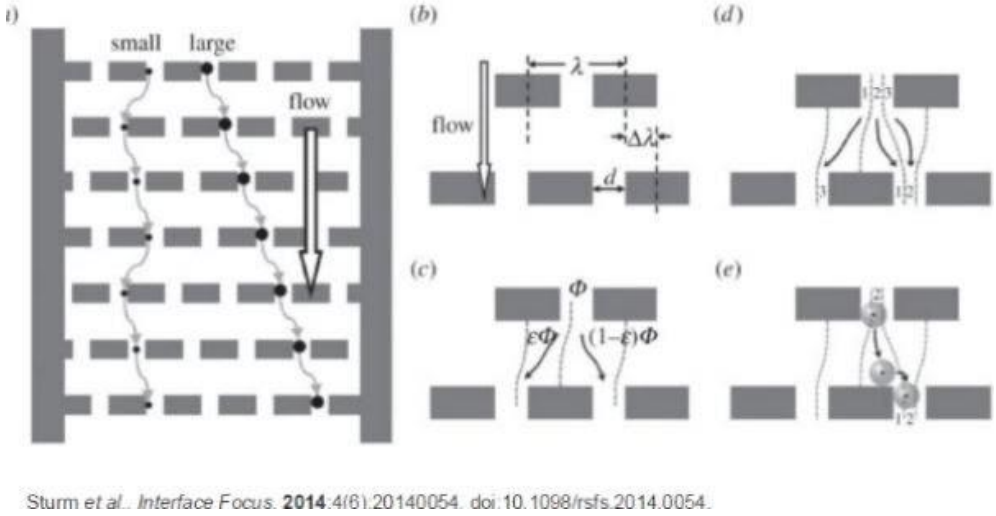
II. Appendix II: Preliminary Designs

Plinko

The Plinko design is inspired by a pricing game from The Price is Right. 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. While the motion appears to be random, a specific set of mathematical and physical principles guide 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 [1]. 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 [2].



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 [3]. 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.

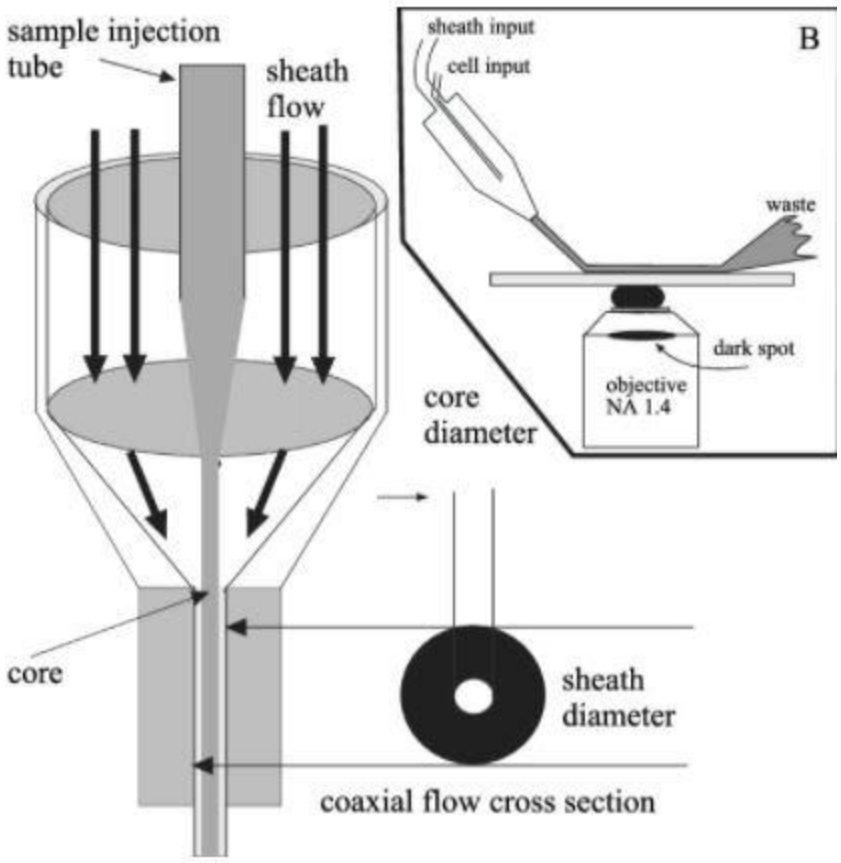


Figure 2: A typical conical shaped funnel design which incorporates angular positioning to bolster fluid speed.

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.

Snake

The main design consideration behind the Snake design is the property of inertial lift. 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. This idea was implemented into a microfluidic device by Di Carlo in 2007, and when an asymmetric design was implemented, a result that bears close resemblance to Figure 3 was produced [4].

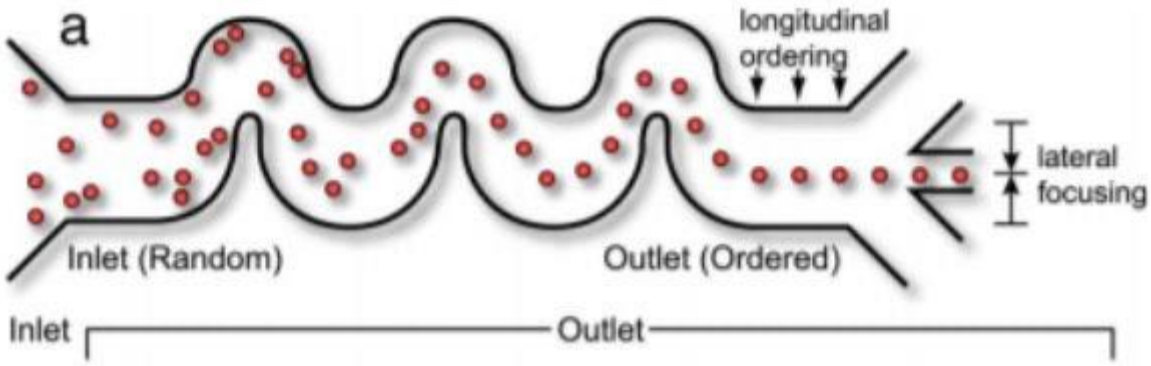


Figure 3: A schematic of a sample prototype for an asymmetrical serpentining channel. The summation of inertial lift forces encourages cells to form a line as they take the path of least resistance towards the outlet.

The snake design was expanded and the concept of inertial ordering was added to a set of potential improvements to cell sorting [5]. 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 [6]. 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.

References:

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[2] 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.

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[6] 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.

III. Appendix III: 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 1: 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.