Microfluidic Cell Sorter
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].

[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].