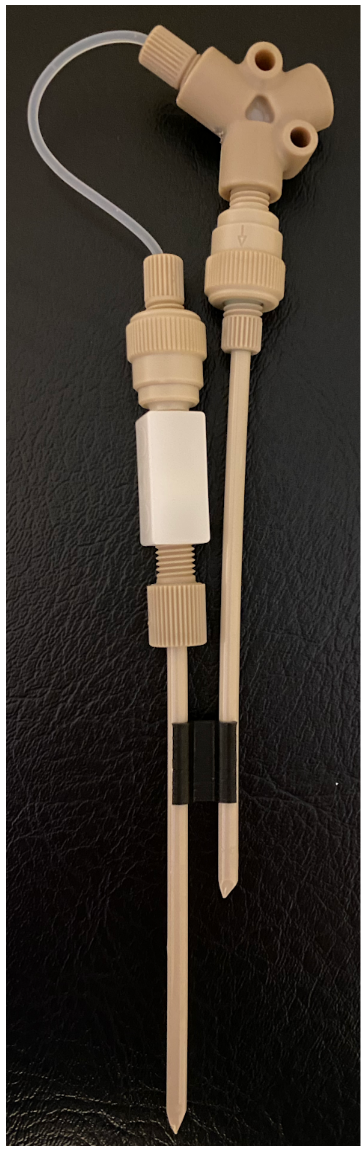
Fluid Cartridge Mixing Strategy

Sponsored by Norman Khoo and Stephanie Ogrey at Illumina



Bryson Pierce | Chen (Derek) Liu | Diego Padilla | Josie Han | Syed Rizvi

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Jerry Tustaniwskyj
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Executive Summary

Genome sequencing has become one of the most important biomedical applications in recent years as worldwide pandemic strikes. Since conducting genome sequencing for such viruses is one of the most useful and straightforward methods of figuring out the source and behavior of these viruses to protect humans, biomedical companies and laboratories around the world have been trying to improve the current method of sequencing. The sponsor of this project, Illumina, is one of the cutting edge biotech companies across the world that is able to conduct genome sequencing onsite or by request for research purposes. Besides onsite operations, Illumina is also providing sequencing machines and cartridges in the market for those who want to conduct genome sequencing at their convenience. Shipping is an important issue for cartridges where the reagents needed for sequencing that come with the cartridge is perishable, this means Illumina has to ship the reagent with a large amount of dry ice to keep its integration during shipping progress. This greatly increased shipping cost and thus entire product cost. Illumina has implemented a substitution shipping method to reduce shipping cost. Instead of ship reagents with dry ice, Illumina dehydrates reagents before shipping and asks customers to rehydrate reagents onsite before conducting any tests. Such a method will inevitably involve mixing multiple reagents with buffers during rehydration, and this project aimed to optimize the current mixing method Illumina is using.

The mixing strategy Illumina currently is using is to implement a sipper connected to a syringe by tubing that is controlled by a syringe pump. The syringe pump aspirates and dispenses fluid from and into the well automatically according to a predetermined recipe. The purpose of this project is to revise this method by applying changes to the system, including the syringe pump, tubing, sipper and reagent well.

As discussed by the team, the availability for modification has been limited into the sipper design. As the objective of this project is to revise the current mixing method, it was inappropriate to change the original structures provided by the sponsor. Changing the sipper design was the most plausible design approach by comparison in table 2.

The team proposed several possible designs, such as “shower head” nozzle, jet nozzle, and the fluidic plate. Each has been put into CFD analysis and physical testing, the result shows that T-Nozzle performs the best by out performing other nozzles by 62.66% when measured by the coefficient of

variation. Furthermore, the proposition of implementing a tesla valve also brought another option for the team. Unfortunately the testing result for tesla valve did not show a promising change in the performance of mixing. Instead, the team decided to use a check valve, which serves the same purpose, and assembled two check valves together to achieve a system that can aspirate fluid from the bottom of the well and dispense at the surface level of the fluid in the well along with the nozzles designed. The new system that circulates fluid inside the well achieved a 290% increase in efficiency when compared to the control nozzle, which accomplished the project's optimization goal.

Abstract

This project, sponsored by biotech company Illumina, aims to develop a novel and efficient mixing process to decrease the time taken to reach a homogeneous mixture between two reagents, increase ecofriendliness, and reduce manufacturing and shipping costs. Within the process of genome sequencing, mixing together reagents is a pivotal step and takes up valuable time. Inefficient mixing processes result in increased sequencing time, increased equipment costs, and ultimately, fewer genomes fully sequenced. To quantify the efficiency of a given mixing process, a test bed consisting of a 6-port pump, Syringe pump, 24-port valve, and two specialized cameras was created. This test bed imaged the mixed solution after a given amount of time, using millions of data points to determine the degree of homogeneity. Using this method, several pumps of varying designs were tested, along with multiple mixing "recipes". The mixing strategy deemed most efficient with consideration to the costs of manufacturing was implementing Check Valve Apparatus. This mixing process was shown to have a coefficient of variation (a measure of inhomogeneity) of 75.75%, which equated to a 290% increase of mixing efficiency from the control.

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Chapter 1: Project Description

Background

In this project, the team aimed to optimize the current robust mixing strategy provided by Illumina. Previously, Illumina had shipped their fluid cartridges with copious amounts of dry ice to keep the reagents at the correct temperature. Due to the adverse environmental effects and high shipping costs caused by this, Illumina has developed a different shipping method, involving the dehydration of reagents into solid powders (lyophilization) before shipping and rehydrating the reagents back onsite. This rehydration process requires the mixing of two or more reagents with different densities and viscosities. Therefore, due to this process, achieving homogeneity between reagents efficiently was the ultimate goal.

Review of Existing Design Solutions

Illumina's existing solution was injecting and extracting fluids back and forth from a well using a standard "sipper" nozzle. This process was repeated several times until the reagents formed a homogeneous mixture. However, this method was very time consuming and unreliable in forming complete homogeneous mixtures.

Some other methods that have been explored by Illumina include magnetic stir bars, which drastically improved mixing performance. However, adding magnetic components and a motor to the system made it difficult to ship, difficult to implement, was costly, and had a negative environmental impact.

The aim of this project was to avoid such problems faced previously by Illumina and provide a mixing strategy that is efficient, cost effective, and easy to manufacture and transport.

Statement of Requirements and Deliverables

The general requirements of this project included:

- Design a mixing strategy that can quickly mix rehydrated reagents homogeneously
    - Optimize nozzle geometry
    - Develop custom "recipe" for mixing
- Small or no changes on basic structures (external well geometry, tubing size, etc.)
- Better mixing than control strategy (pumping back and forth with standard sipper)
- Keep Design for Manufacturing (DFM) in mind
- Low cost
- Ease of transportation
- Environmentally friendly

The final deliverables to the sponsor were:

- One (1) prototype of all developed components of the final design, which:
    - Mixes more efficiently than the “control” strategy.
- Bill of Materials
- Scripts and mixing “recipes” used
- CAD files/drawings of all relevant designs
- Fully documented report including methods used, designs paths explored, reasoning behind design decisions, and quantitative measurements of effectiveness.

Chapter 2: Description of Final Design Solution

Although the purpose of this project was to quantify the effectiveness of numerous mixing strategies to aid in the future development of Illumina's genome sequencing platforms, one specific design rose above the others: the check valve apparatus. This design reached the greatest level of homogeneity with relatively low production/implementation costs. In fact, it reached an average Coefficient of Variation (CoV, a measure of inhomogeneity, lower is better) of 75.75%. This is a great improvement from Illumina's existing sipper nozzle, which achieved a CoV of 219.30%.

This design is inspired by one of the major goals of this project, which was to propose an alternative method of mixing that is considered different from the current existing method of implementing one sipper for both aspiration and dispensation. A possible way of refining such a method was to use two separate channels for aspiration and dispensation, but the problem was the difficulty to connect two sippers with single tubing and also fit in the inlet of the well.

For application purpose, the aspiration nozzle could be any nozzle as in fact there is no significant difference in mixing ability during the aspiration process. The dispensation nozzle could be any nozzle including custom nozzles, since the nozzles generally show a more significant effect of mixing during the dispensation process.

For comparison purposes, the team decided to test and assemble the check valve apparatus using two default control nozzles from Illumina before implementing custom nozzles as outlets.

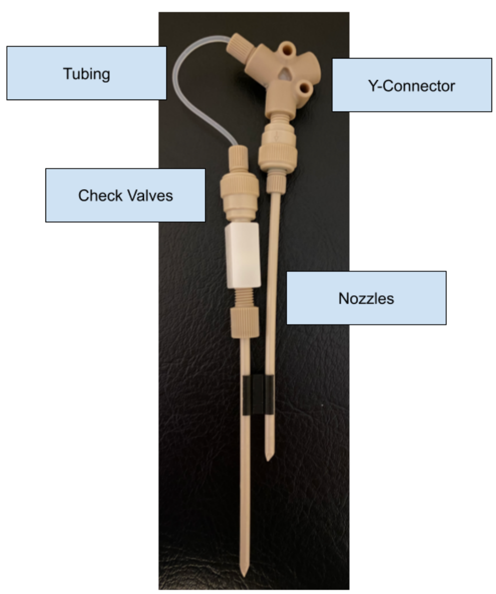

Figure 1 : Check Valve Apparatus

As seen in **Figure 1**, this design is the Check Valve Apparatus. It was able to obtain a 75.75% CoV. When compared to the control's CoV of 219.30%, this was a 290% increase in mixing efficiency. Detailed analysis of this design is discussed in Chapters 3 and 4.

This design aimed to solve the main problem that caused incomplete mixing, the sedimentation of reagent. By taking fluid from the bottom and dispense to the top surface level, it is introducing the reagent that could not dissolve at previous dispensations to a redissolve process and spreading across the entire well, instead of only dissolving inside the nozzle and spreading only at the bottom level of the fluid, which could still cause sedimentation of the reagent. By dispensing at the top level of the fluid inside the wall, it is giving more time for dissolution and more surface contact between the reagent and the buffer solution, which thus increases efficiency of dissolution.

Chapter 3: Design of Key Components

Within this project, there were two categories of components designed: those for the test bed, and those for the mixing strategies. Key components designed by the team are listed below:

- Test Bed
  - Camera Mount
  - Tubing Mount
  - Post-Mixing Aspiration Nozzle
  - Nozzle Hub
- Mixing Strategies
  - Jet Nozzle
  - Shower Head Nozzle
  - Tesla Valve
  - Check Valve Apparatus
  - T-Nozzle
  - Propeller Balls

However, these were not the only components of the system. Equipment provided by Illumina, as well as equipment purchased from industry suppliers, was used in the project. All of these, along with the team's custom designs, are detailed below:

Test Bed Components

The design of the test bed was especially important, as this would be used to quantify the effectiveness of each and every design. The test bed was created with equipment supplied by Illumina, custom 3D printed mounting designs, and other third-party equipment. The major components of this setup are listed below, and illustrated in **Figure 2**:

1. Buffer and reagent solutions
   - The solutions to be mixed together.
   - The buffer solution had a volume of 171.4 mL and was primarily composed of distilled water. The reagent solution had a much smaller volume of 4.6 mL and was more dense and viscous than the buffer solution. Because of this, the reagent tended to settle at the bottom of the well and had to be agitated in order to mix with the reagent and achieve homogeneity.
2. Mixing well

- Where the mixing happened. In each test, it began pre-filled with the buffer solution. Then, the reagent solution was deposited before the mixing process began.

3. 6-port syringe pump equipped with a 5 mL glass syringe
    - Primary mechanical driver of the system. Connected to a laptop via USB in order to programmatically conduct mixing “recipes”.
4. 24-port valve
    - Central hub for the system; controlled where the fluid was directed, between the syringe pump, reagent reservoir, and the mixing well. Connected in tandem with the 5 mL syringe pump to the laptop via USB to conduct mixing recipes.
5. Three reservoirs for the following fluids:
    - Reagent
        i. As mentioned previously, this was one of the fluids to be mixed. Before testing, this was kept in a small reservoir, waiting to be deposited into the mixing well.
    - Manipulation fluid
        i. Instead of pushing and pulling air in the syringe pump, an incompressible fluid (water) was used instead, in order to create more consistent pressure differences and flow rates.
    - Waste
        i. Whenever fluid had to be ejected from the system (such as making room for aspirated reagent or well mixture), it was deposited into the waste reservoir.
6. Monochrome camera equipped with a high-power blue LED laser and green light filters
    - Primary data acquisition method; Taking pictures of the fluid after the mixing process at a constant acquisition rate to determine the level of homogeneity. Further detail on this process is provided in Chapter 4.
7. A high capacity syringe pump equipped with a 100 mL plastic syringe
    - After mixing was complete, this syringe pump aspirated the well volume at a constant rate of 20 mL/min. This allowed the camera mentioned above to view a unique section of fluid for every image.
8. Tubing and related fittings
    - 3.175 mm (1/8”) OD, 1.5875 mm (1/16”) ID tubing.
    - 1.5875 mm (1/16”) OD, 0.762 mm (0.03”) ID” tubing.
    - 6-40 flat bottom fittings and ¼”-28 flat bottom fittings for 1.5875 mm (1/16”) OD tubing.
    - ¼”-28 flat bottom fittings for 3.175 mm (1/8”) OD tubing.

An annotated figure with these components is included below:

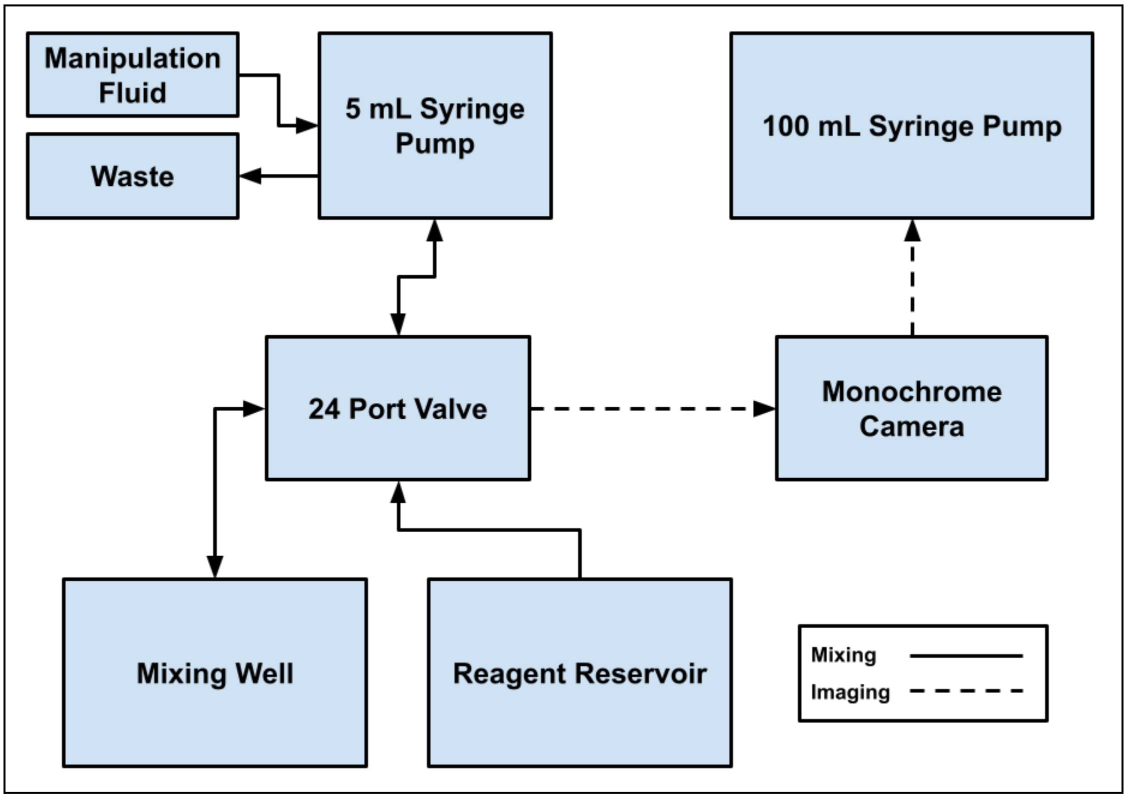

Figure 2: Test bed used for quantification of mixing strategy effectiveness.

This test bed diagram in Figure 2 illustrates the pathways the fluid traveled during the testing process. First, mixing is done within the solid pathways, using the 5 mL syringe pump to drive the system. After mixing is complete, the fluid pathway is switched from the 5 mL syringe pump to the 100 mL syringe pump, allowing the monochrome camera to image the fluid as it is aspirated. This process is further detailed in the “Testing Methodology” section in Chapter 4.

Camera Mount

Although the monochrome camera was provided by Illumina, a mount to keep the camera upright and steady was absolutely necessary. It was decided that a 3D printed mount that would couple the camera to a heavy metal mounting plate (also provided by Illumina), would be ideal. A technical drawing of this mount is included below in Figure 3.

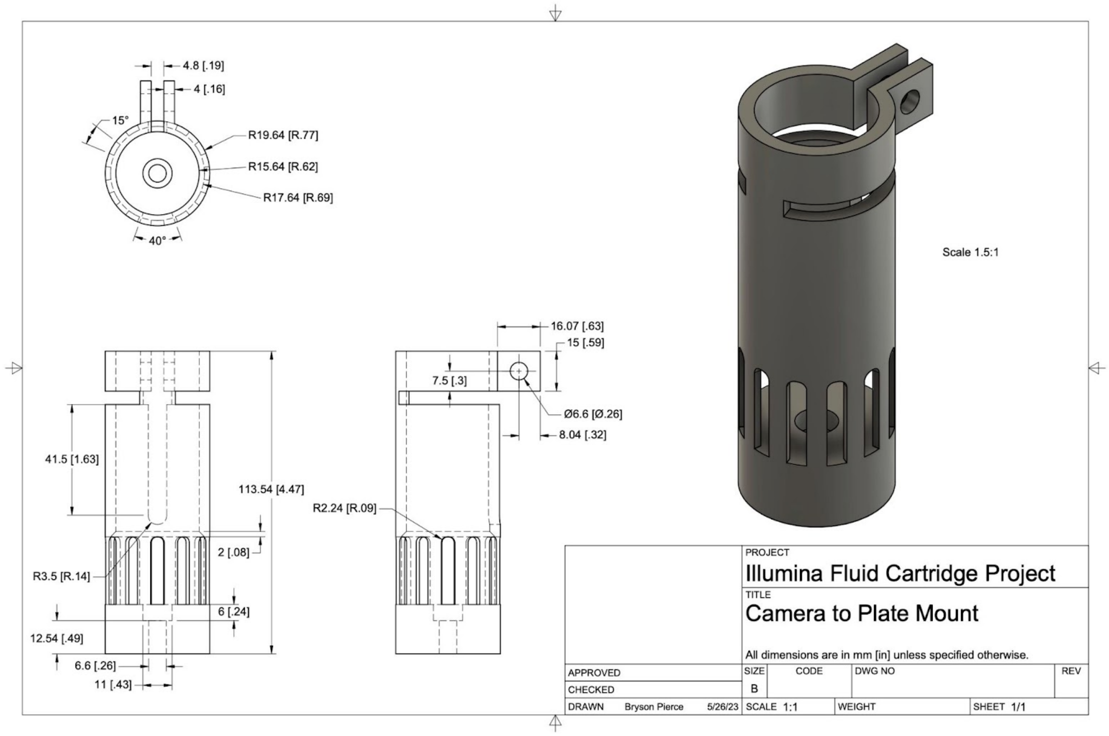

Figure 3: Technical drawing of custom "Camera to Plate Mount". Can be seen in clearer detail in Appendix C.

Tubing Mount

The tubing mount was designed to keep the tubing in the same position relative to the camera at all times. It mounted directly to the filter cage on the camera, and was secured with 4-40 screws.

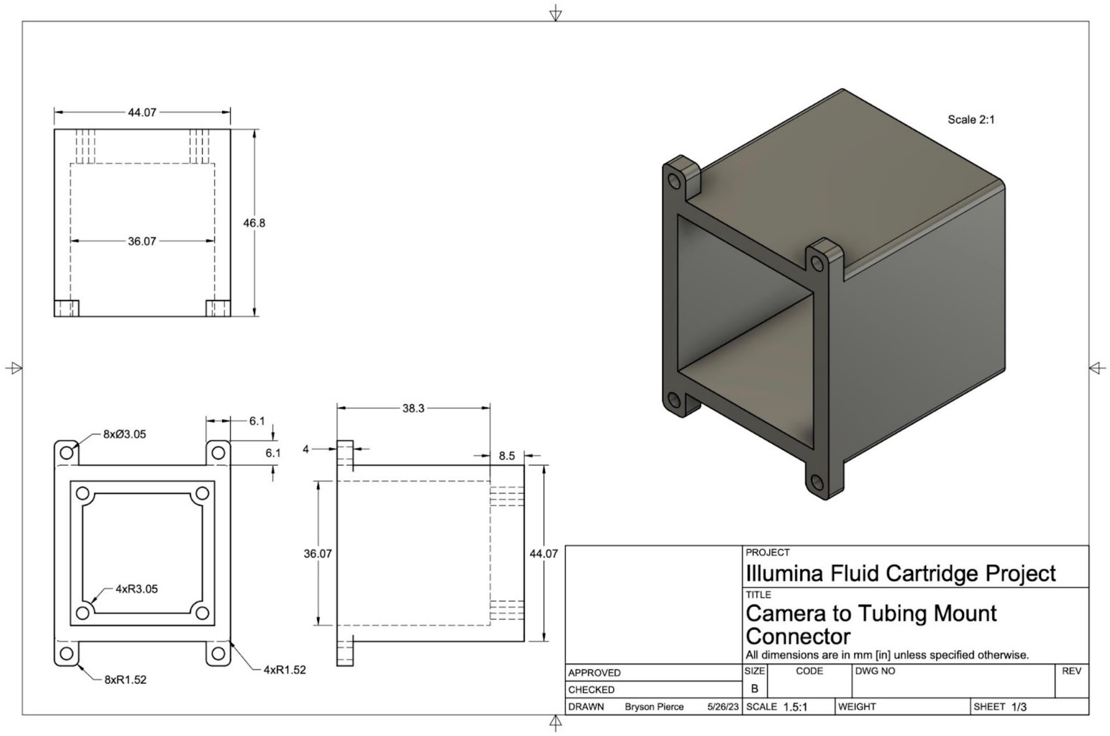

Figure 4a: Technical drawing of the connector component in the tubing mount. This connected the filter cage of the camera directly to bottom plate of the mount, as drawn in Figure 4b, as well as providing a dark environment for imaging. Can be seen in clearer detail in Appendix C.

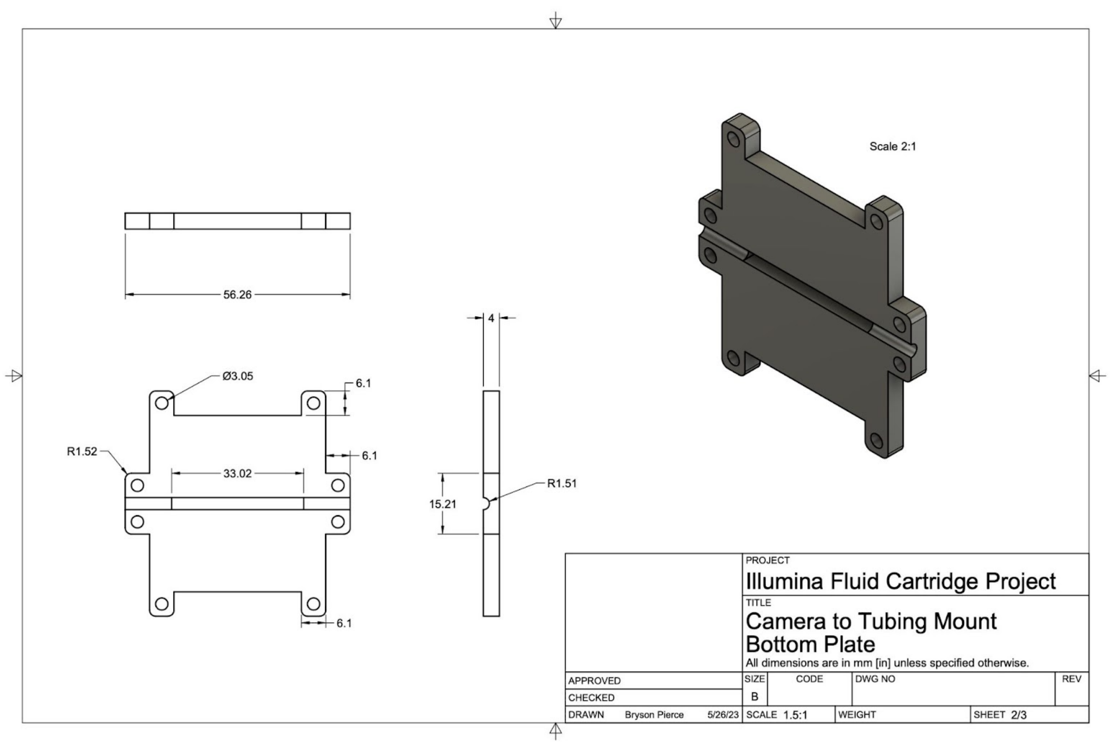

Figure 4b: Technical drawing of the bottom plate component of the tubing mount. This connected to the "connector" component and clamped the tubing with the top plate, as drawn in Figure 4c. Can be seen in clearer detail in Appendix C.

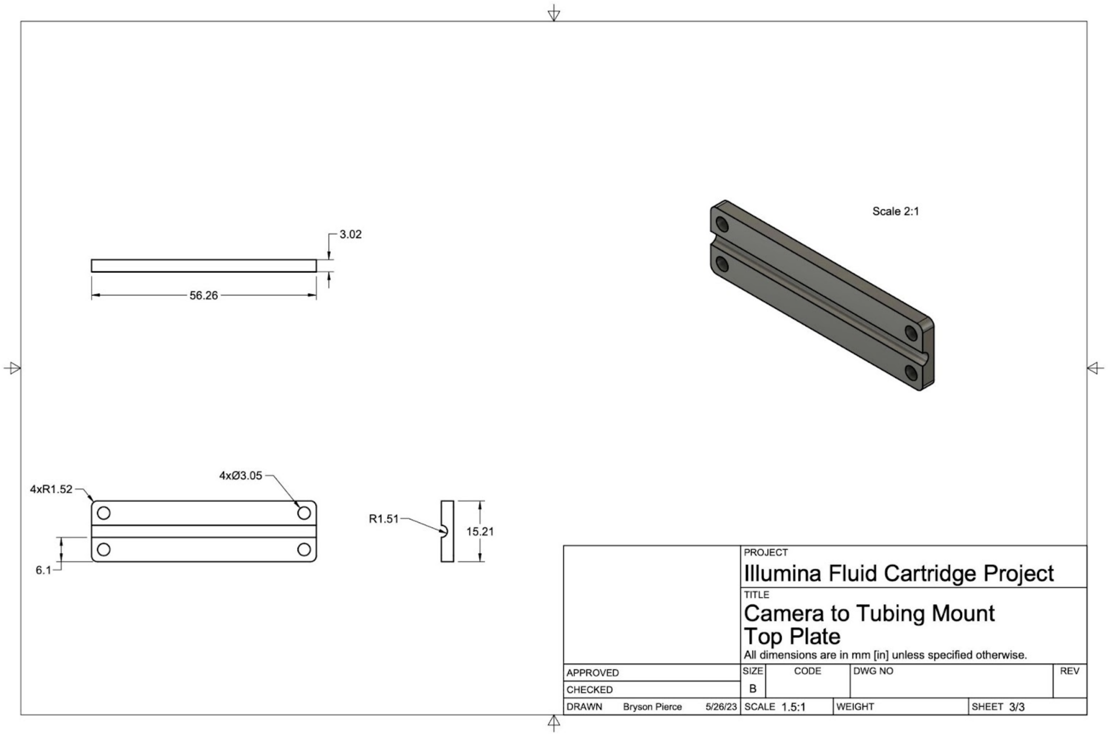

Figure 4c: Technical drawing of the top plate component of the tubing mount. This clamped the tubing with the bottom plate in Figure 4b. Can be seen in clearer detail in Appendix C.

Post-Mixing Aspiration Nozzle

The 3D polyjet printed aspiration nozzle was a long nozzle with a larger inner diameter that was designed to be used after the mixture was complete. The nozzle would aspirate all 171.4 mL of mixed reagent over 3 passes at a constant flow rate of 20 mL/min since the syringe had a max capacity of 80 mL.

Figure 5: Aspiration Nozzle, meant to aspirate the well volume after applying mixing recipe.

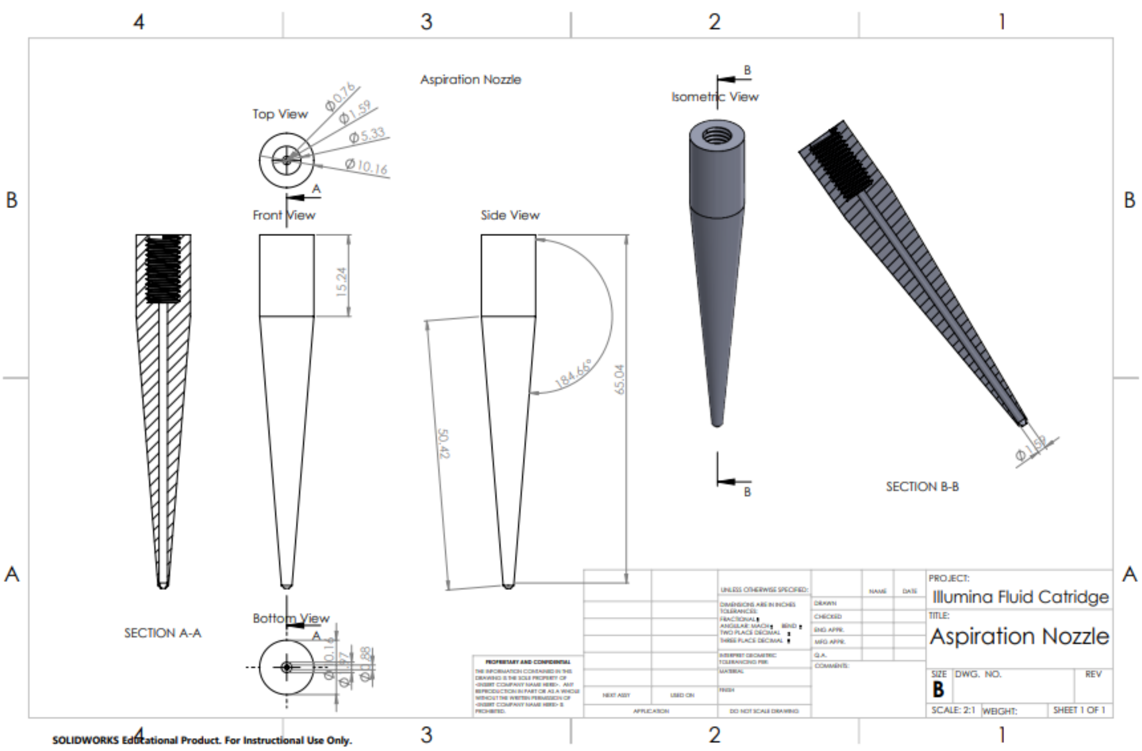

Figure 6: Technical drawing of Aspiration Nozzle with multiviews. Detailed view can be seen in Appendix C.

Nozzle Hub

A cylindrical hub was initially designed to provide a connection for several nozzles. It provided a rigid connection to any type of nozzle such as the shower head, jet, and aspiration nozzle with the rest of the system during and after the mixing process. It provides connection of the tube from the system to the nozzles using ¼”-28 threaded holes and 1.5875 mm channel.

Figure 7: Nozzle Hub. The hub has two ¼”-28 threaded holes with a 1.5875 mm channel. Provides a modular bottom connection to several nozzles (i.e. Shower Head or Jet)

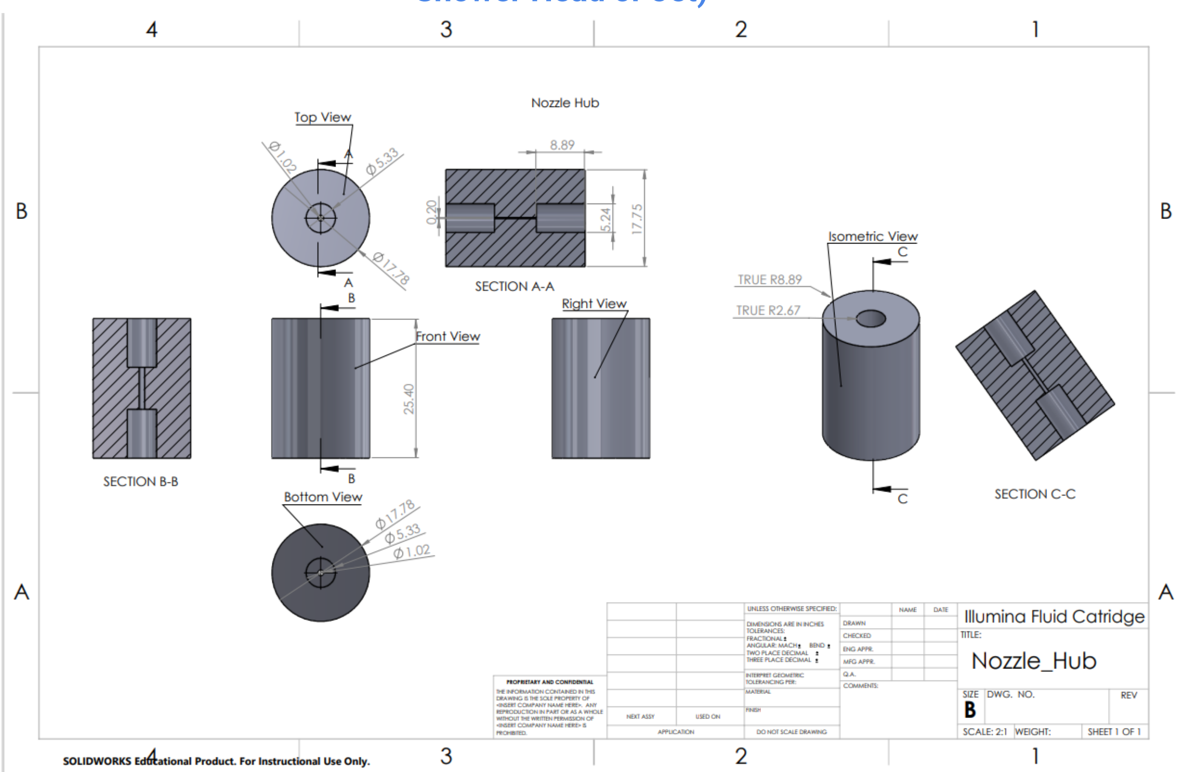

Figure 8: Technical Drawing of Nozzle Hub with Multiviews. For detailed drawing refer to Appendix C

Mixing Strategies

Design Process

During the beginning of the design process, the team brainstormed a key design direction. The following tables and Pugh charts were created to decide mixing strategies to pursue.

In Table 1, possible mixing strategies are listed for this project. Magnetic Mixers involve using a stir bar and magnetic drive to stir the fluid present in the well. Motor Mixers consist of a mechanism similar to coffee stirrers where the motor spins the stirrer in the cup. Both Magnetic and Motor mixing strategies were complex to integrate with the test bed system and proved to be costly. Sippers were decided to be the best solution due to having the lowest costs and were less complex in suiting the team's design purpose.

	Magnetic Mixer	Motor Mixer	Sipper
Complexity	Medium	High	Low
Manufacturability	Medium	High	Medium
Cost	Medium ($50-300)	High ($500+)	Medium (Based on Design)
Other Concerns	Environmental Costs	Circuit Integration with software	Too Slow

Table 1 : Mixing Strategy Comparison

MIXING STRATEGY:		Magnetic Mixer	Motor Mixer	Sipper	Weights of the Row
total number of components	highscore = less components	1	1	3	2
number of off the shelf parts		3	3	1	3
number of custom parts		2	1	1	3
cost of ordered parts	highscore = less expensive	1	2	3	2
max lead time for ordered parts		2	1	3	2
complexity of custom parts		1	1	2	3
time to fabricate		2	2	1	2
number of systems that must be adjusted		1	1	3	2
points of failure (possible leaks? jamming?)		1	3	2	1
mixing time for _ volume of liquid		2	3	1	3
time needed to manufacture custom parts		1	1	2	2
environmental impact		2	2	1	2
resulting coefficient of variance (how nonhomogeneous is the mixture?)		1	1	2	3
volume of footprint (how compact can we make it?)		1	1	3	1
Total Score		49	51	58	

Figure 9 : Pugh Chart for Mixing Strategies. Several mixing strategies were scored on several key factors. The higher the score the better. Each row was weighted based on the impact it had on the strategy. Magnetic Mixers scored the lowest at 49 points, while the Sipper scored the highest at 58 points.

The Pugh Chart for mixing strategies was a chart that listed all the important factors for three different types of mixing strategies. A magnetic mixer was a subsystem that used magnets to rotate and mix the reagent inside the well. Illumina had previously attempted using magnetic mixers. And although this type of design gave promising results, it was at the bottom of the team's list due to environmental and transportation costs. A motor mixer was a type of mixer that required a motor to perform the mixing of reagents. Even though the motor mixer scored higher than the magnetic mixer, it was not by a lot. Therefore, the team decided to not pursue this type of mixing strategy since it was too complex but was open to it if time permitted at the end. Sippers scored the highest on the Pugh chart and supported the team's previous decisions.

Discussions for possible modification locations in the system were also made. As the sponsor suggested, the team should not change or have only small changes to the well geometry since changing well geometry will force the sponsor to change the geometry of the entire cartridge to accommodate for such changes.

After evaluation, the team had decided to modify sipper and tubing only as they are easy to modify and would not result in further modifications in other systems to accommodate for the changes made in this project.

	Sipper and Tubing	**Well Geometry**	**Well Lid**	**Syringe and Pump**
Implementation Difficulty	Low	Medium to High	Medium to High	High
Benefits	Medium	Medium	Low	Low
Availability	Recommended	Possible	Possible	Not Recommended

Table 2 : Comparison of Design Modification Availability

It was determined that further design direction for the project would be modifying sippers and tubing without making changes to the rest of the strategy.

Shower Head Nozzle

Some of the connections that were attached at the end of the hub (described in Test Bed Components) included, a Shower Head Nozzle, and Jet Nozzle. The shower head nozzle was first designed with the intention that as fluid passed through the chamber and exited through the outlet holes, turbulent mixing would occur within the well itself. The final design of the shower head nozzle can be seen below in **Figure 10**.

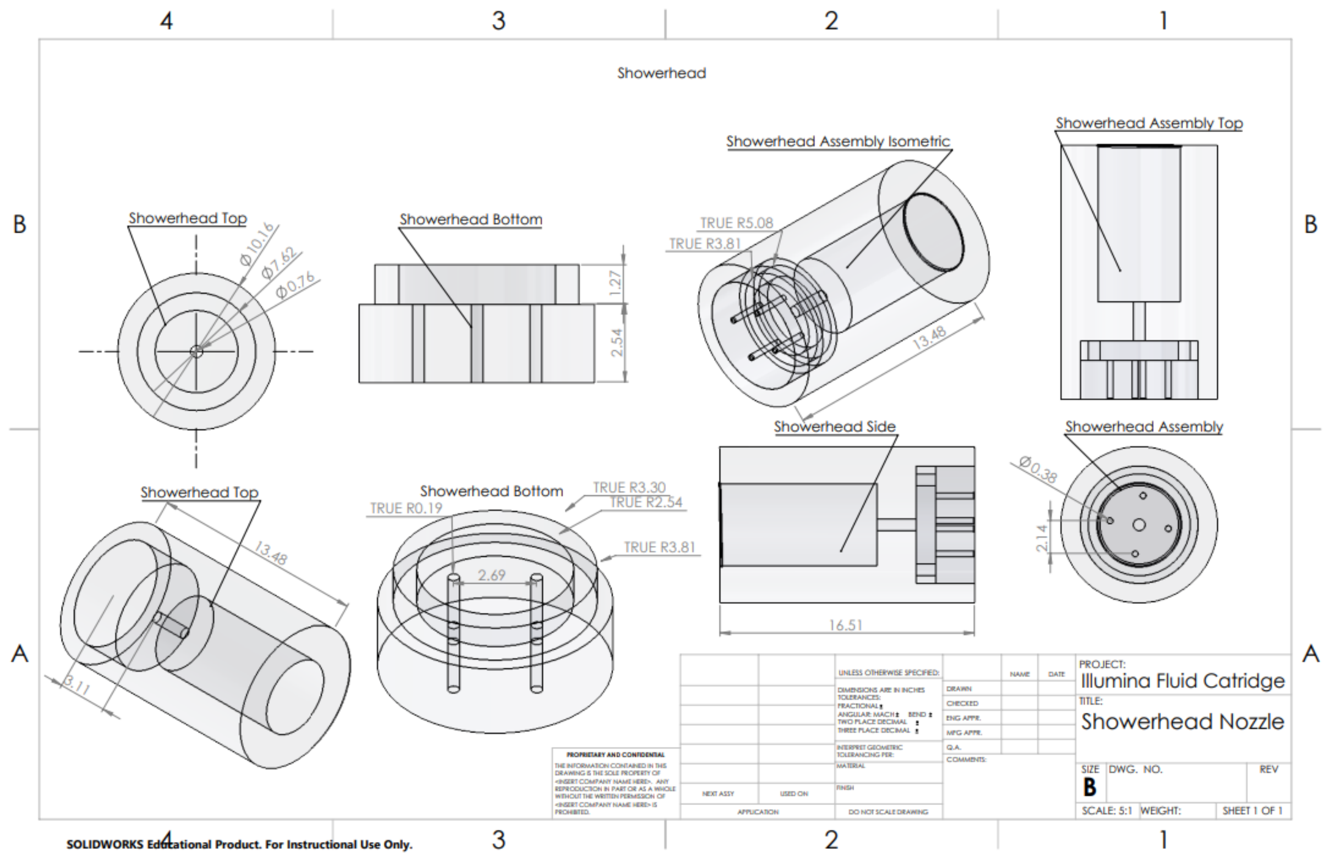

Figure 10 : Technical drawings of Shower Head Nozzle (mm). It consists of a circular bottom with 4 outlet holes with a diameter of 0.762 mm, a chamber circumference by the bottom, and the inlet base with tapered threads. Refer to Appendix C for detailed view.

The fluid passes through tubing (OD: 1.5875 mm, ID: 0.762 mm) and into the hub. From there it slides down to the sipper. After passing through the sipper, fluid is ejected out into the well.

To quickly verify whether our assumptions for the shower head nozzle were accurate, simple CFD simulations were created to visualize the mixing of fluids within the design. As shown in **Figure 11** most of the mixing appeared to be occurring just before entering the chamber region [green].

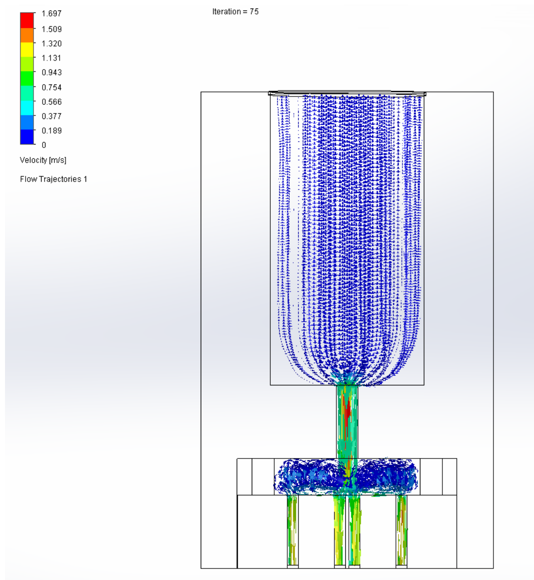

Figure 11 : CFD Simulation of Shower Head Nozzle.

As fluid accelerates by flowing into the small channel, mixing is created. The high speed fluid crashes onto the bottom wall of the chamber before flowing out through the outlet holes.

To test whether this design was actually feasible, real tests needed to be conducted, despite manufacturing issues at such a small scale. This part was able to be fabricated through Tormach CNC Lathe.

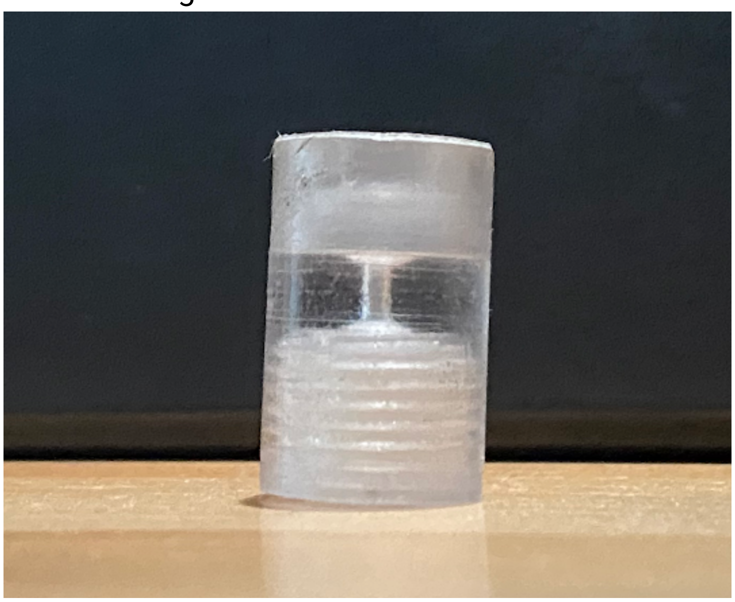

Figure 12 : Shower Head Nozzle manufactured through Polyjet Printer (Material: VeroClear) and Manual CNC (Material: HDPE).

Once the shower head nozzle was prototyped. The completed part was added to the test bed and followed the mixing strategy described in Chapter 3.

Jet Nozzle

Another nozzle that was designed to be connected at the end of the base was the Jet Nozzle. The Jet Nozzle is different from the shower head nozzle, with only one outlet hole. Using the speed at which the fluid exited the nozzle, turbulent mixing would occur within the well itself. Below is the final design of the Jet Nozzle with its appropriate dimensions.

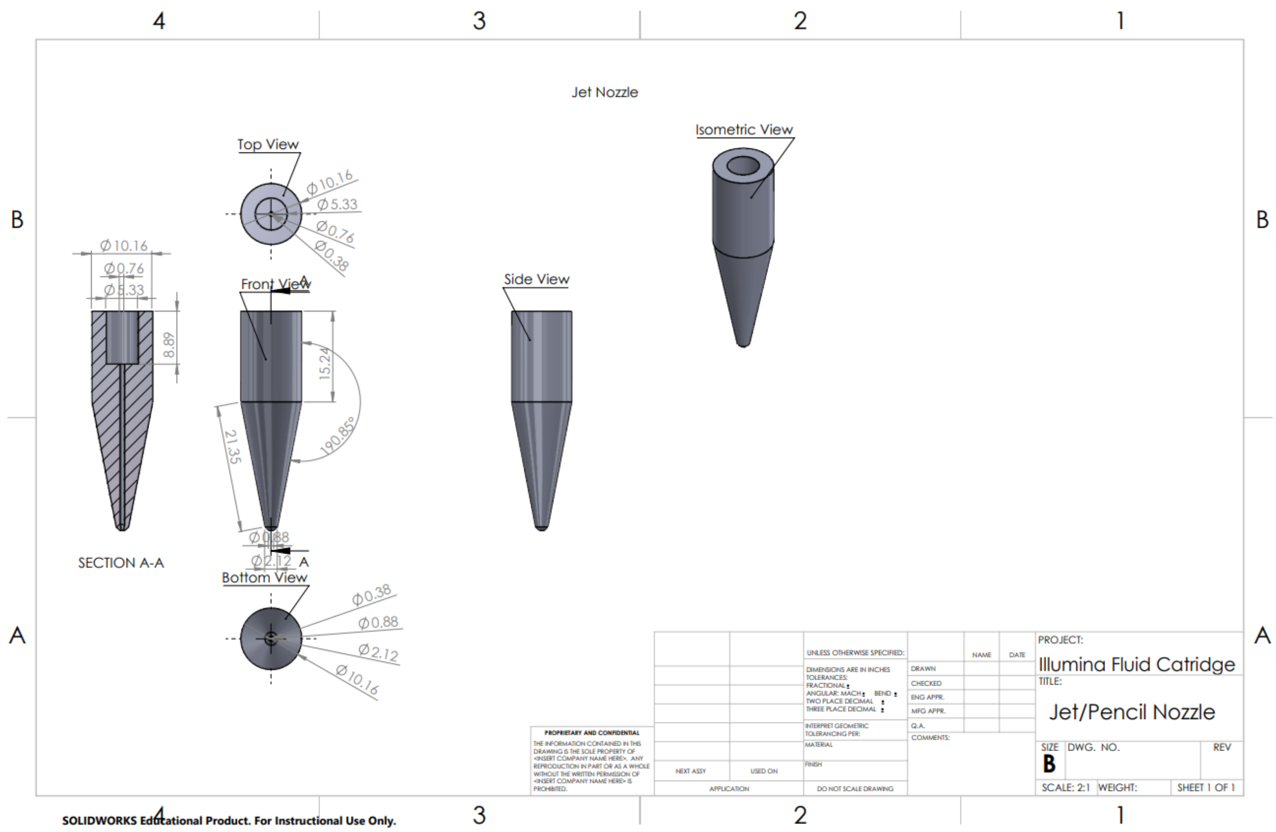

Figure 13: Technical Drawing of Jet Nozzle (mm). The jet nozzle has a thin (0.762 mm) and a long (25.4 mm) channel inside the ¼ "- 28 threaded hole. The outlet is tapered with a diameter of 0.38 mm. The tubing (OD: 1.5875 mm, ID: 0.762 mm) is connected to the jet nozzle via fittings consisting of a pair of ¼"-28 nut and ferrule.

Fluid flows from the tubing and through the nozzle at the same velocity until reaching the tapered outlet. The outlet reduces the area and increases velocity of the fluid, allowing a wider dispense spray as it exits through the nozzle [1]. This property allows the reagent to mix quicker and thoroughly in the well as compared to a conventional straight sipper nozzle. The jet nozzle can operate independently or in combination with other nozzles.

Simulations were also created for the Jet Nozzle to test how mixing would occur from within. Figure 14 shows the results of this simulation.

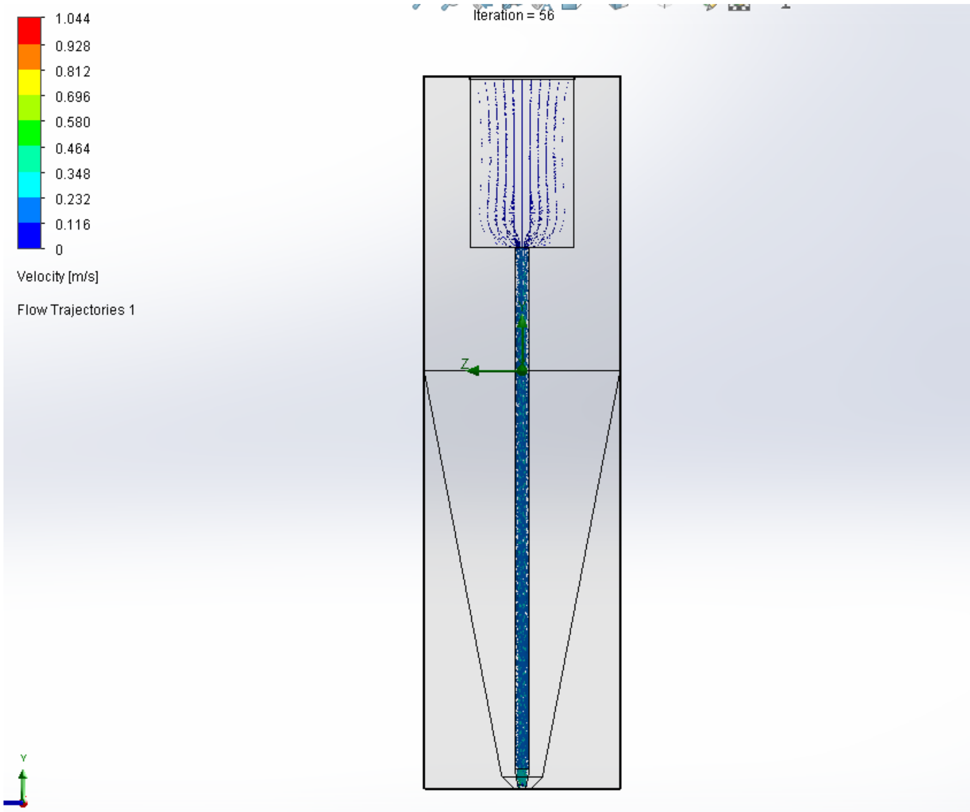

Figure 14: CFD Simulation of Jet Nozzle.

The CFD Simulations showed little to no internal mixing, however there was a slight increase in the outlet fluid velocity due to the change in diameter. To prove whether this change in velocity would help with mixing within the well, the jet nozzle was fabricated through Tormach CNC Lathe.

Figure 15 : Jet Nozzle manufactured through Tormach CNC Lathe.

Fluidic Slide

The fluidic slide was initially designed to have two inlet tubing connections at the ports and one outlet connection. A chamber with unique geometry would be implemented at each of the ports before intersecting at a junction. However due to the volume difference between buffer and reagent, this design was dropped since no real significance in mixing would be achieved. Manufacturing a complex device like this would prove to be difficult and expensive as well.

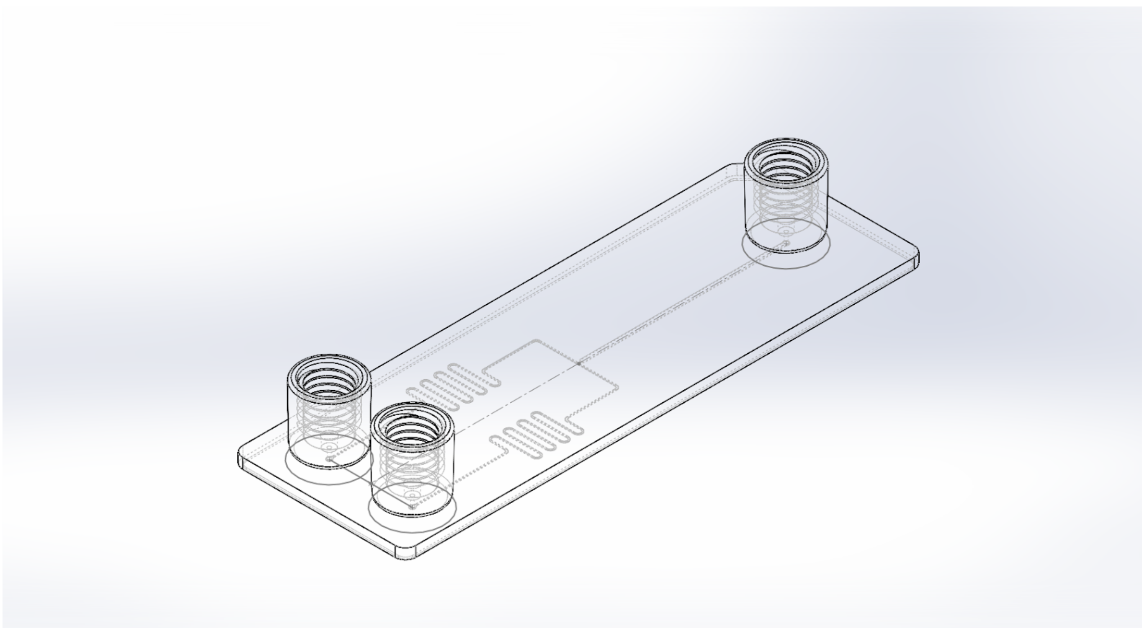

Figure 16: CAD design of Fluidic Slide. It has 2 inlets, 1 outlet (¼ 28-Ports), 100 micron inlet channels, 400 micron wide outlet channels, a nozzle region (T-junction), and a thermal Cap. Fluid enters the 100 micron channels before converging at a junction that is 400 microns wide. The intersection of the fluid should help with mixing, as it then exits the device through an outlet port.

Tesla Valve

Other designs were also explored, such as the Tesla Valve. The Tesla Valve was able to be imitated at a much smaller scale. It was designed so that fluid would only be allowed to flow through one direction and nearly zero flow from the opposite direction. Fluid would flow smoothly from top to bottom while dispensing and bottom to top during aspiration. The turbulent flow created when fluid passing through this valve would induce thorough mixing. Figure 17 displays the final CAD of the Tesla Valve before being manufactured through Tormach CNC.

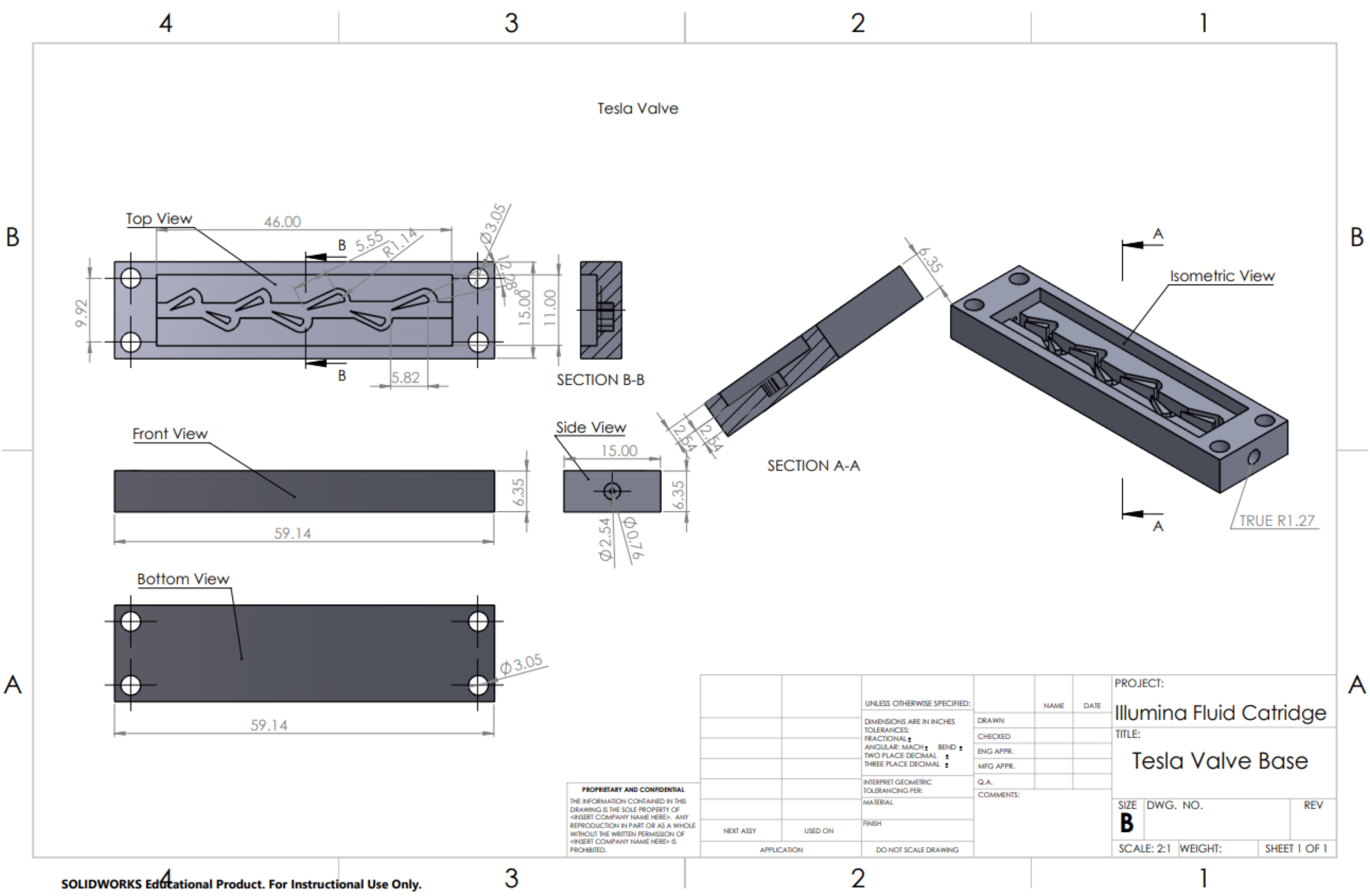

Figure 17: Technical Drawing of Tesla Valve with multiviews (mm). It consists of a main channel and several small channels separated by triangle obstacles. Refer to Appendix C for detailed view

The Tesla Valve underwent the same verification process and the results are shown in **Figure 18a** and **Figure 18b.**

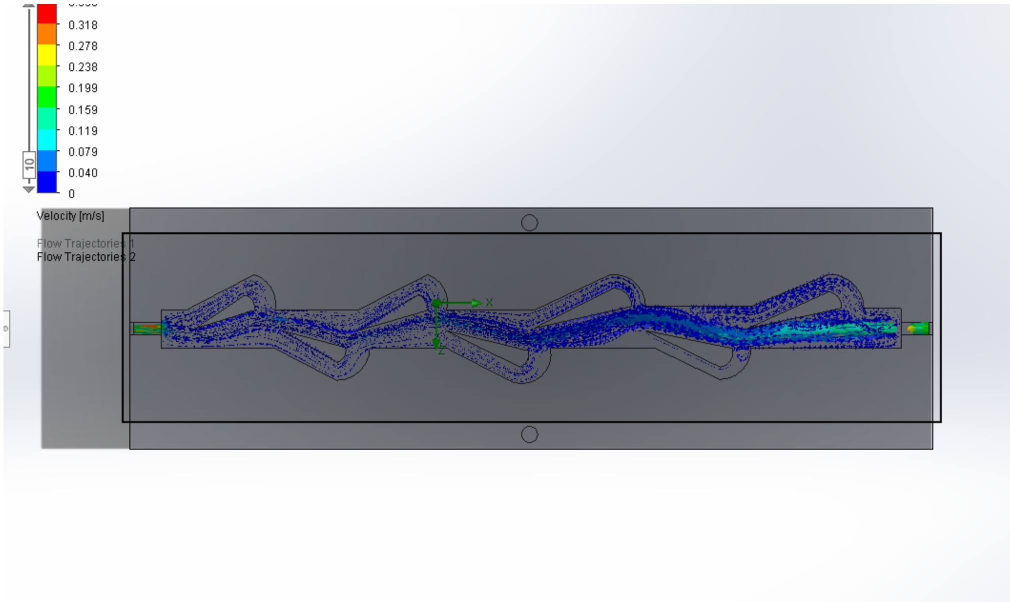

Figure 18a: CFD Simulation of Tesla Valve with fluid flow inlet at the bottom. Fluid is flowing in the smooth direction which has high velocity at the beginning and having no problem flow across the structure.

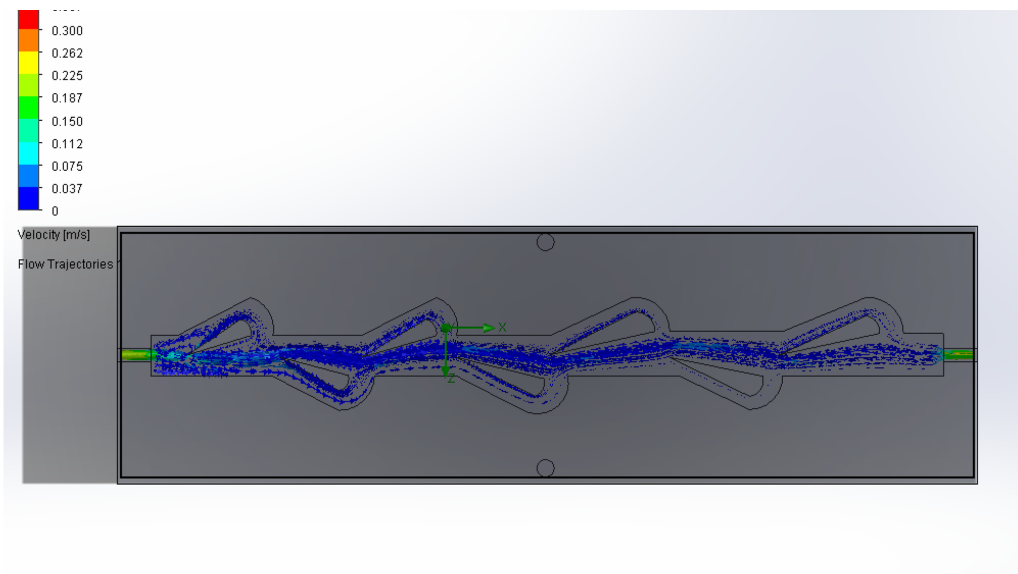

Figure 18b: CFD Simulation of Tesla Valve with fluid flow inlet at the top. Fluid is flowing in the resistance direction which results in a slower initial and overall velocity compared to previous simulation.

It was realized that the Tesla valve's ability to resist flow in a single direction was not as effective as hoped. Although a non-mechanical option would be very useful, it wasn't practically feasible. For this reason, off-the-shelf check valves were then pursued.

Check Valve Apparatus

The Tesla valve's intent of one-way flow without mechanical parts was promising, but not possible in the end. Check valves, on the other hand, can provide a secure, sealed, one-way flow.

One of the biggest problems faced by the project was the settling of the denser reagent at the bottom of the well since reagent has a considerably larger density than the buffer that results in sediments. There was no way for a typical nozzle to pull the reagent from the bottom of the well to the top of the well without a complex motorized system to automatically change the nozzle height. Instead, the team decided to separate the system into two channels with different abilities and height. This design would create a circulatory check valve system that aspirated fluid from the bottom of the well and dispensed fluid at the top as seen in the schematic, **Figure 19**.

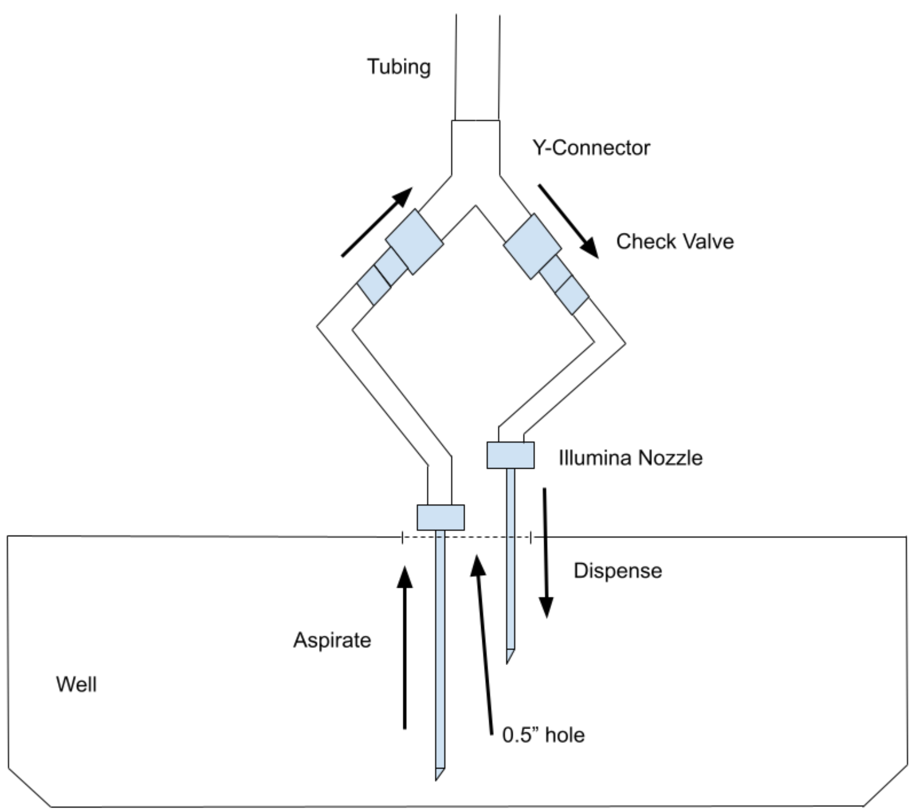

Figure 19 : Schematic of full Check Valve Apparatus. Tubing connects to a Y-connector. Two check valves attached to the Y-Connector control the direction of flow of two nozzles, one which dispenses near the top of the well, and another which aspirates near the bottom of the well.

Check valves provide a secure one-way flow. By splitting the fluid line with the Y-connector, in conjunction with two check valves, one could achieve a dual-nozzle system without the complications of multiple fluid lines and pumps. In this case, the aspiration and dispensation process is separated but can still be operated by a single syringe pump.

This design, pictured in **Figure 1,** can aspirate fluid from the bottom of the well and then dispense fluid to the top surface level of the fluid in the well. A tube clamp was implemented to hold the two nozzles together so it would be compact enough to fit in the small inlet hole of the well, and also be able to adjust the vertical distance between the bottoms of the nozzles. For the purposes of this project and the well provided, the distance between the bottoms of the nozzles is about 38.1 mm (1.5 in) to reach a maximum vertical distance between the bottom of the well and top surface level of the fluid inside the well.

This design incorporated several components. This includes one Y-connector, two check valves, two nozzles, along with the measured tubing.

Figure 20: Y-Connector with ¼“-28 thread on female port

Figure 21 : In-line Check Valve with ¼“-28 thread on male port

These components were ordered together to ensure a consistent ¼” - 28 thread in the entire apparatus and compatible with the rest of the test setup.

Propeller Balls

Lastly, to help further agitate the fluid and by request from the sponsor of implementing structures inside the well, small propeller balls were added to the system. **Figure 22** shows the final design of the Propeller balls:

Figure 22 : Propeller Balls manufactured through polyjet printer (Material : VeroClear)

This design was designed to sink to the bottom of the fluid in the well. These propeller balls were expected to be agitated by the fluid flow from the nozzle pushed onto the fins during the dispensation process. Due to this expectation, the balls were designed to have density large enough to sink at the bottom, where the nozzles generally dispensed fluid, to achieve maximum efficiency. Note that due to this nature of the design, propeller balls are not supposed to be implemented at the same time with the Check Valve Apparatus.

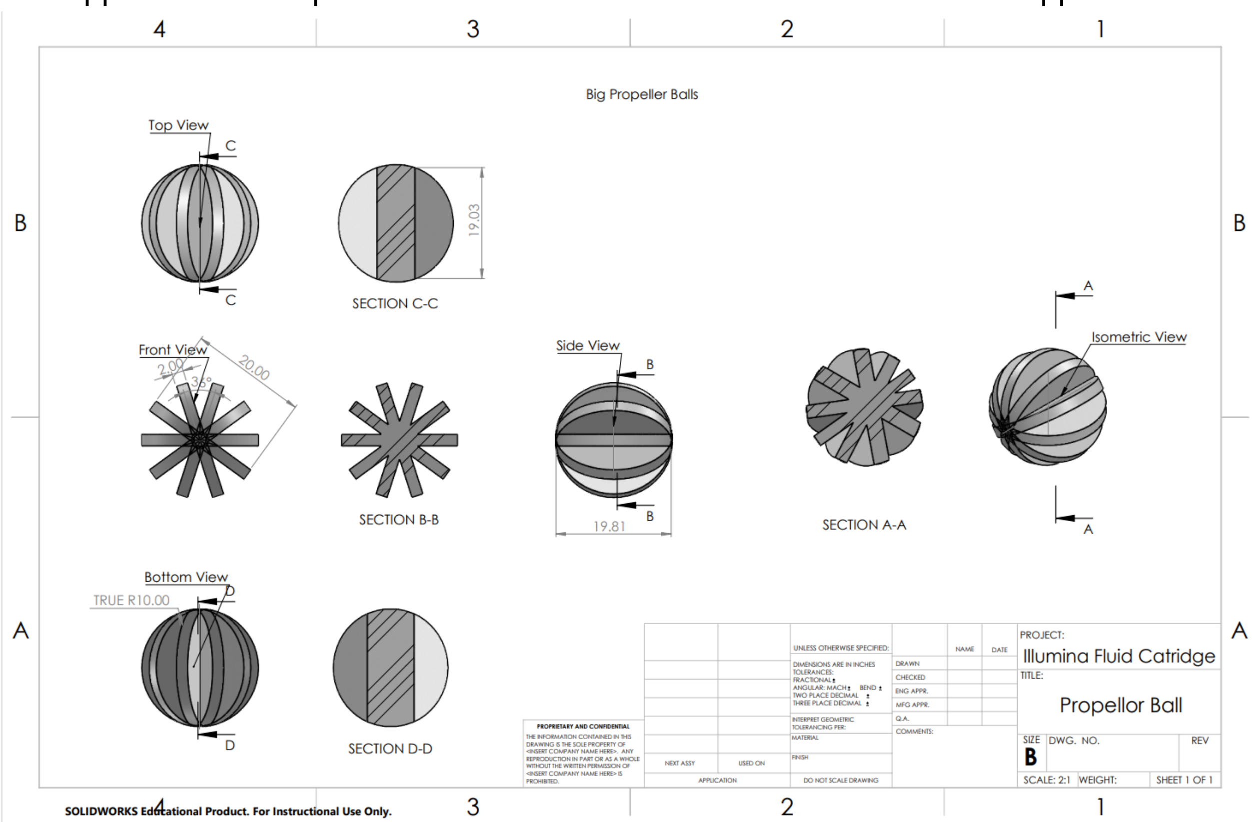

Figure 23: Technical Drawing of Propellor Balls with multiviews (mm). Refer to Appendix C for detailed view

T - Nozzle

This nozzle has a long and thin vertical channel for accelerating the fluid, and two horizontal outlets to dispense fluid out at the same altitude. The name came from the shape of its internal channel that is shaped like a reversed letter T.

This nozzle is originally designed for an augmentation of the Check Valve Apparatus. It is supposed to be implemented as the dispensation nozzle for Check Valve Apparatus due to its ability to spread the fluid out in the same height, which would be the top surface level of the fluid as expected for Check Valve Apparatus. The team later decided to test this design individually since it also showed a promising ability of improving mixing.

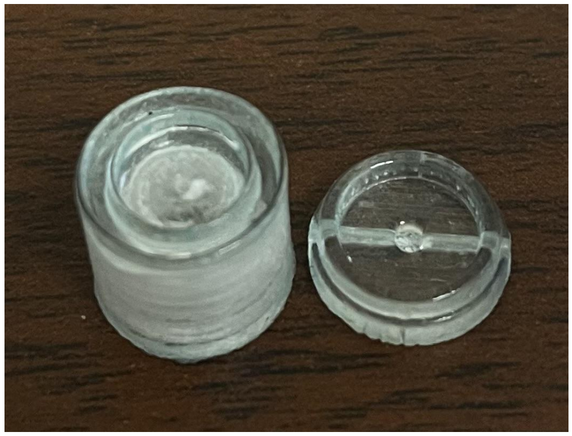

Figure 24 : T-Nozzle prototyped using polyjet printer (Material : VeroClear)

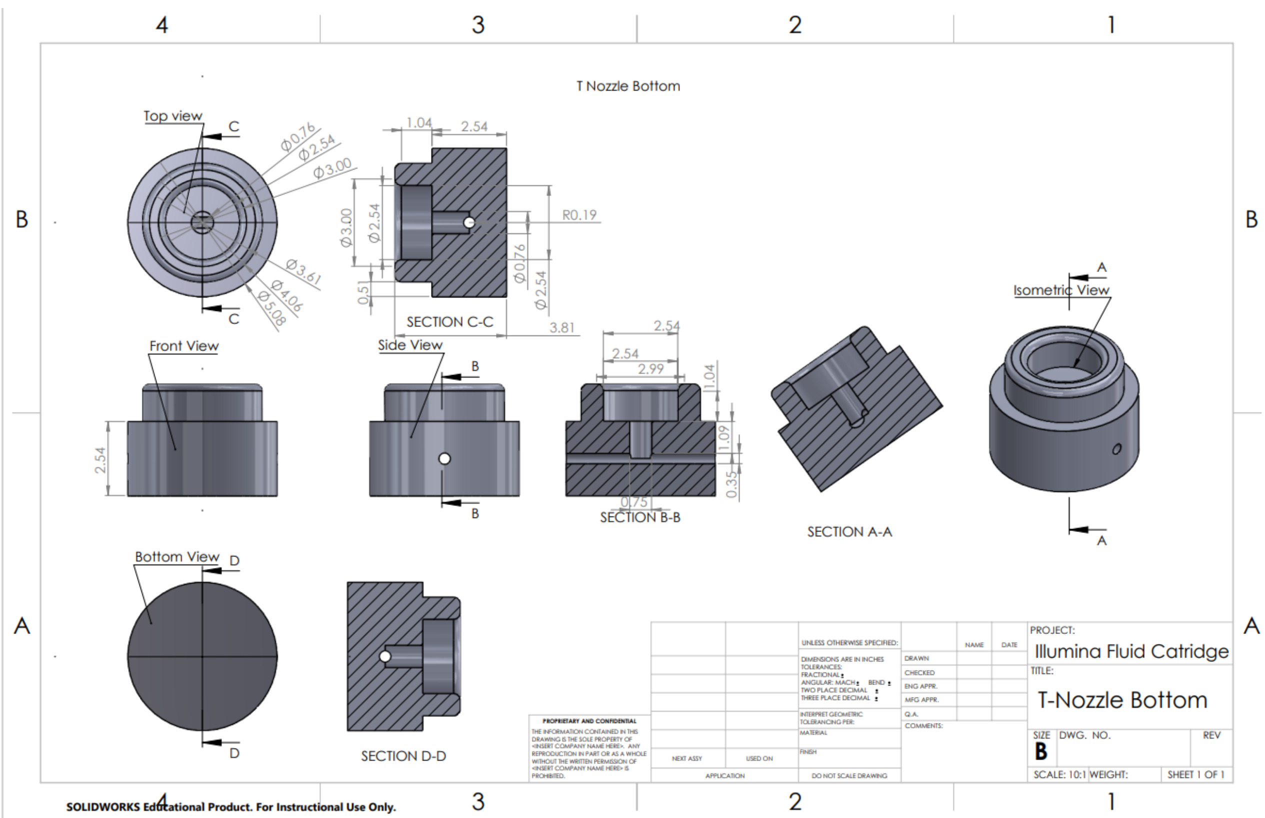

Figure 25a : Technical drawing of the internal view of the T-nozzle bottom. Refer to Appendix C for detailed view

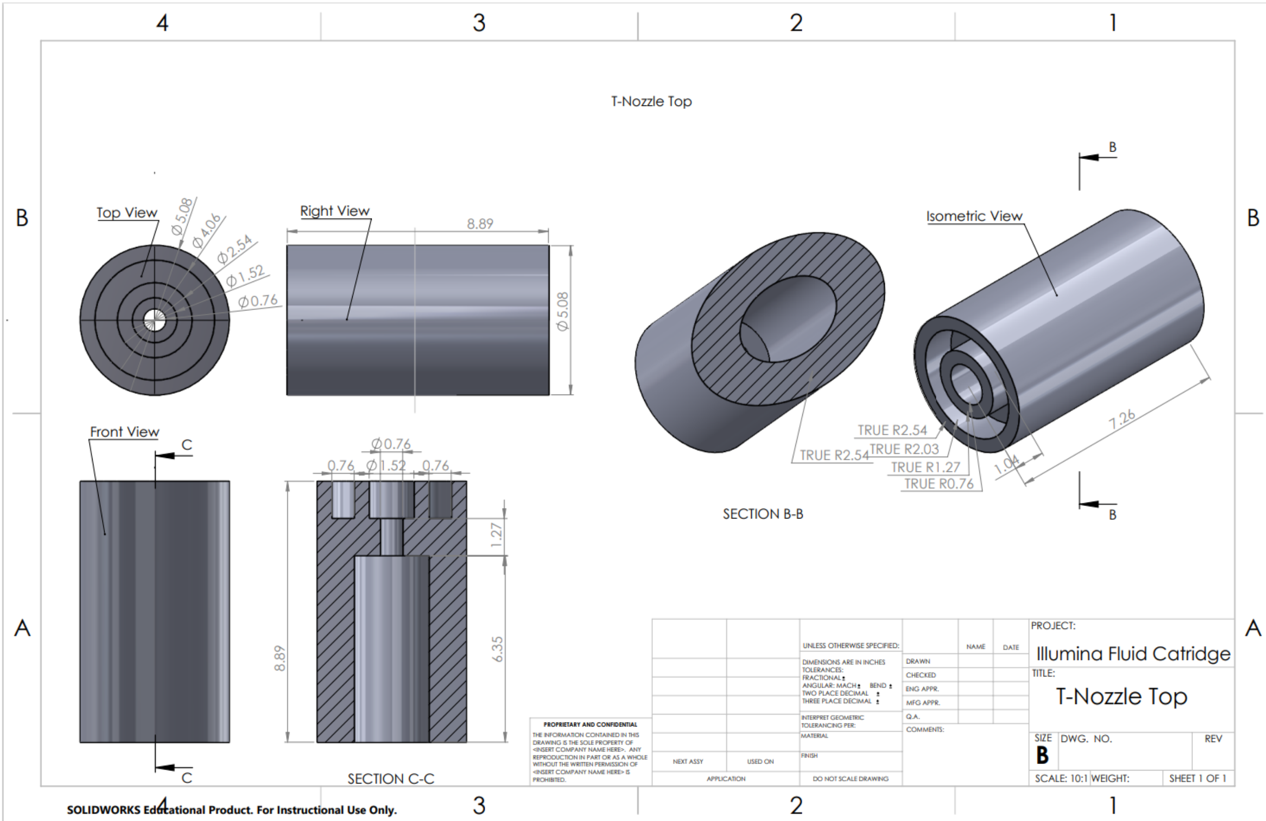

Figure 25b : Technical drawing of the internal view of the T-nozzle top. Refer to Appendix C for detailed view.

Chapter 4: Prototype Performance

Testing Procedure

The assembly of a capable test bed was only the first step in creating an effective testing process. In order to generate accurate quantitative results for each mixing strategy, a robust and precise testing procedure was followed. The testing procedure was composed of three phases: Pre-mixing, mixing, and post-mixing. These three phases are detailed below:

Pre-Mixing Phase

- The buffer and reagent solutions were prepared:
    - Buffer: 171.4 mL of 99.9% distilled water, 0.1% Polysorbate 20.
    - Reagent: 4.6 mL distilled water, 33 µg fluorescein (FSCN) powder, 92 mg Bovine Serum Albumin (BSA) powder.
- The buffer solution was deposited into the mixing well, and the reagent was deposited into the reagent reservoir.
- Any unique nozzle/mixing equipment was applied to the test bed.
    - For all tests, the inlet of the nozzle was always placed as low into the well as possible, without impeding fluid flow.

Mixing Phase

This phase was done entirely automatically, through a programmed mixing "recipe". These mixing recipes can control all aspects of the 5 mL syringe and 24-port valve, including the flow rate, active valve ports, and aspiration/dispensation volume. The team's final mixing recipe did the following:

- Aspirated from the manipulation fluid reservoir and dispensed into the waste reservoir. This primes the manipulation fluid line, removing all air from this part of the system.
- Filled the main fluid line (between the 5 mL syringe and 24-port valve) with manipulation fluid, leaving a small section of air at the end to prevent contamination of the mixing fluids with the manipulation fluid.
- Aspirated reagent from its reservoir, and dispensed it into the mixing well. Due to volume restrictions, this had to be done three times.
- Pushed a small amount of manipulation fluid through the main line to ensure all aspirated reagent was deposited in the well.
- Aspirated fluid from the mixing well, before dispensing it back into the well. This is the primary mixing action and is done a total of five times for every mixing strategy.

All aspiration and dispensation during the mixing phase occurs at 4 mL/min and 6 mL/min respectively, unless stated otherwise.

Post-Mixing Phase

After mixing, the main fluid line is disconnected from the 5 mL syringe pump and reconnected to the high-capacity syringe pump. The mixed solution within the well was then slowly aspirated at 20 mL/min with this pump. As this was done, the solution inside the tubing was imaged by the monochrome camera until the entire mixed solution had been imaged. These images were then used to quantify the effectiveness of the tested mixing strategy, as detailed in the following section.

Data Analysis

To understand the data analysis process, one must understand the properties of the test bed solutions:

- The reagent solution (4.6 mL) contains fluorescein (FSCN), a powder that glows green when dissolved and excited with blue light.
  - Pure reagent appears as white through the FSCN sensitive camera.
- The buffer solution (171.4 mL) does not contain FSCN.
  - Pure buffer appears as black through the camera.

When imaging the fluid, the camera captures the intensity (brightness) of the fluorescent fluid. Brighter means more reagent, whereas darker means less reagent. Perfect homogeneity would mean that each image has the exact same intensity. Therefore, to quantify the homogeneity, or rather, the inhomogeneity, one would want to measure the relative dispersion of the data set. A good indicator of this is the Coefficient of Variation (CoV).

$$CoV = \frac{\sigma}{\mu} = \frac{Standard\ Deviation}{Mean}$$ **Equation 3**

As shown in the above equation, the CoV is simply the standard deviation of the data set divided by its mean. To find this using the images taken by the monochromatic camera, the brightness of each pixel in an image is averaged to make every image a single data point: its overall intensity. Then, these data points are considered as part of the complete data set. The standard deviation and mean of this data set are calculated, which then yields the CoV. Because CoV measures the dispersion of the data set, lower is better. Therefore, the goal of this project is to find a mixing strategy with a noticeably lower CoV than the control strategy.

Results

Results Summary

Shown below is a figure containing the results of every tested mixing strategy.

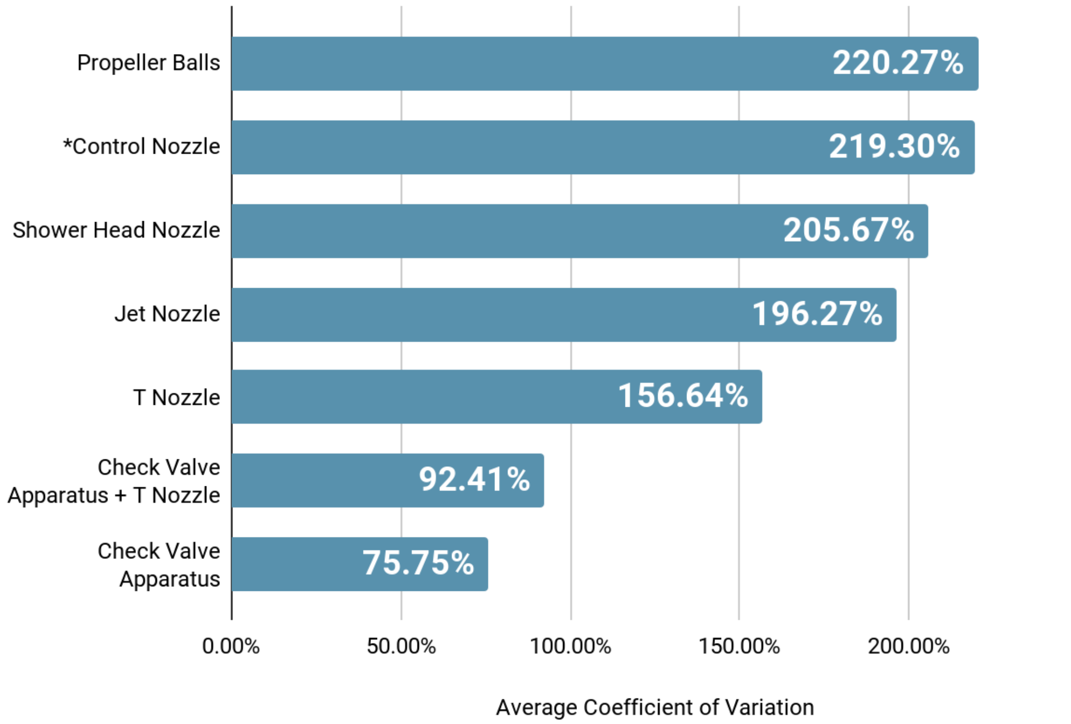

Figure 26: Summary of test results of each mixing strategy. Coefficient of Variation (CoV) measures the dispersion of the data set, so a lower CoV is better.

It can be observed that the control nozzle reached a CoV of 219.30%. The Check Valve Apparatus performed the best, with a CoV of 75.75%, a vast improvement over the control. Using **Equation E4**, it can be calculated that this translates to a 290% increase in efficiency, or nearly a 4x improvement. Propeller Balls performed the worst, with a high CoV of 220.27%. It should be noted that some of these mixing strategies are easier to implement than others, so a slight difference in CoV does not necessarily designate an outrightly "better" design. This is further discussed in Chapter 5.

Control Nozzle

Testing the control nozzle was especially important, since this would be the benchmark to improve upon. Because this benchmark was so pivotal, three tests were conducted exactly as described above, placing the control nozzle at the bottom of the well. The results of these tests are shown below in **Figures 27a-c**.

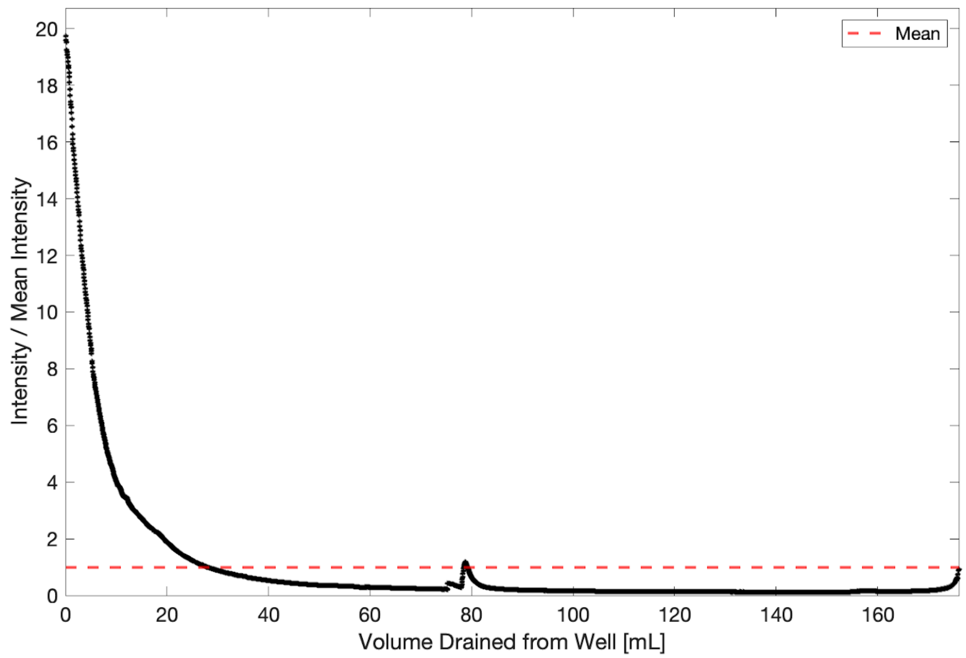

Figure 27a: Control Nozzle, Trial 1, Coefficient of Variation = 254.03%

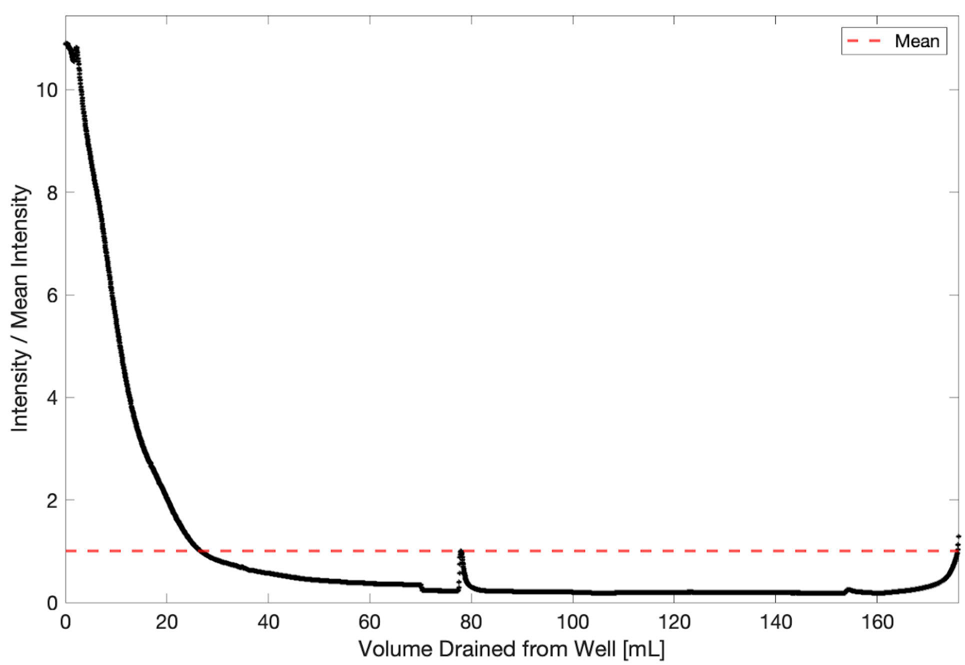

Figure 27b: Control Nozzle, Trial 2, Coefficient of Variation = 206.72%

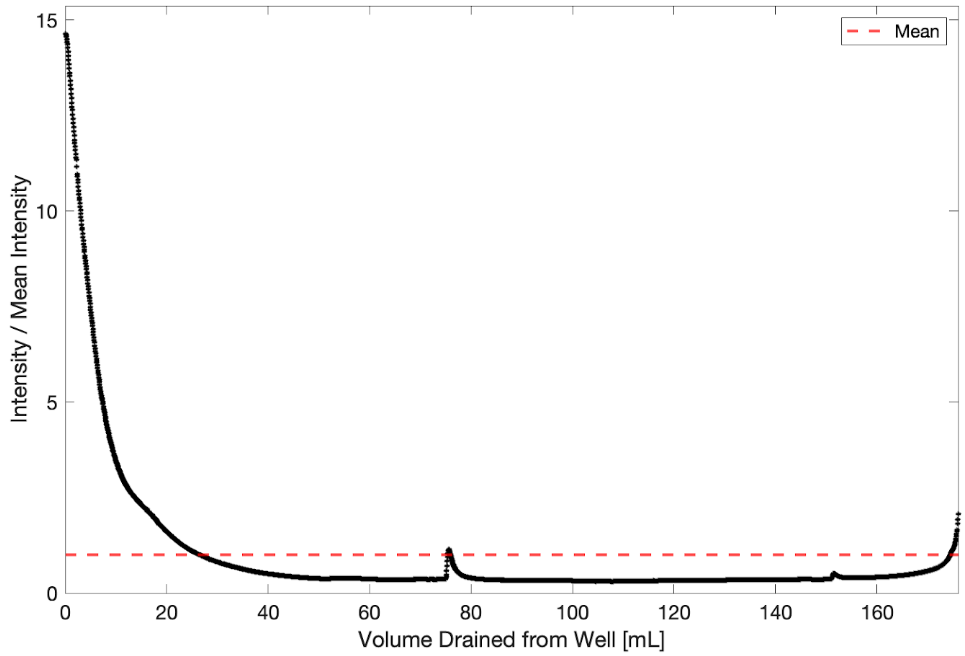

Figure 27c: Control Nozzle, Trial 3, Coefficient of Variation = 196.79%

These tests provide an average CoV of **219.30%**, the number to improve on with the team's designs. The graphs above show the intensity of each image over the volume drained from the well. It can be observed that the greatest intensity occurs at the very beginning. This is because the dense, bright reagent sinks to the bottom of the well, and therefore gets aspirated first. There is a steep dropoff very early in the aspiration process, reaching a fraction of the peak brightness by just 20 mL out of the 176 mL to be aspirated. A faster dropoff correlates to a higher CoV, and therefore worse mixing performance. Optimally, a successful design will have a slower decrease, or a less steep slope. It will also have a low peak relative intensity, as shown on the y-axis. For example, in Figure 27a, the data shows that the fluid reaches a maximum intensity of approximately 20x the mean, which is incredibly high. Decreasing this number, along with the CoV, is the goal of the project.

Additionally, to document the effects of higher flow rates, one test with the control nozzle at 30 mL/min aspiration and dispensation rates was performed. The results of this test are shown below:

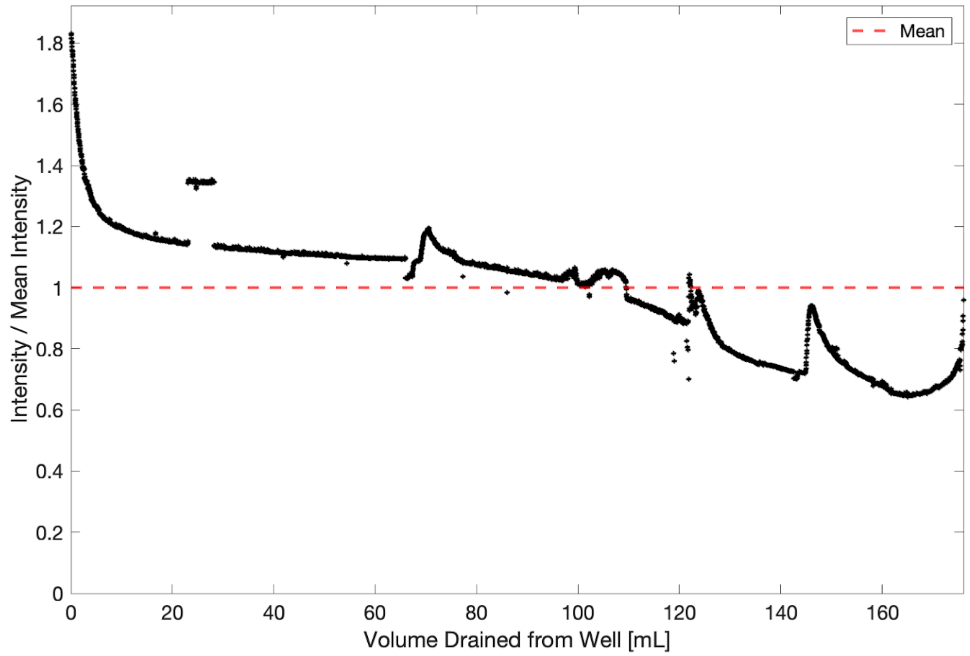

Figure 28: Control Nozzle, 30 mL/min, Coefficient of Variation = 19.59%

This test yielded excellent results, with a CoV lower than any other test. However, as can be seen by the noisy, somewhat unclear data, increased flow rates can introduce other issues, such as bubble formation and high pressures on the system. Although this is not useful as far as a final design, it proves that increased flow rates substantially increase the level of mixing.

Jet Nozzle

The next mixing strategy to test was the team's custom jet nozzle, the most similar to the control strategy of Illumina's sipper. The results of the jet nozzle test are illustrated below in **Figure 29**.

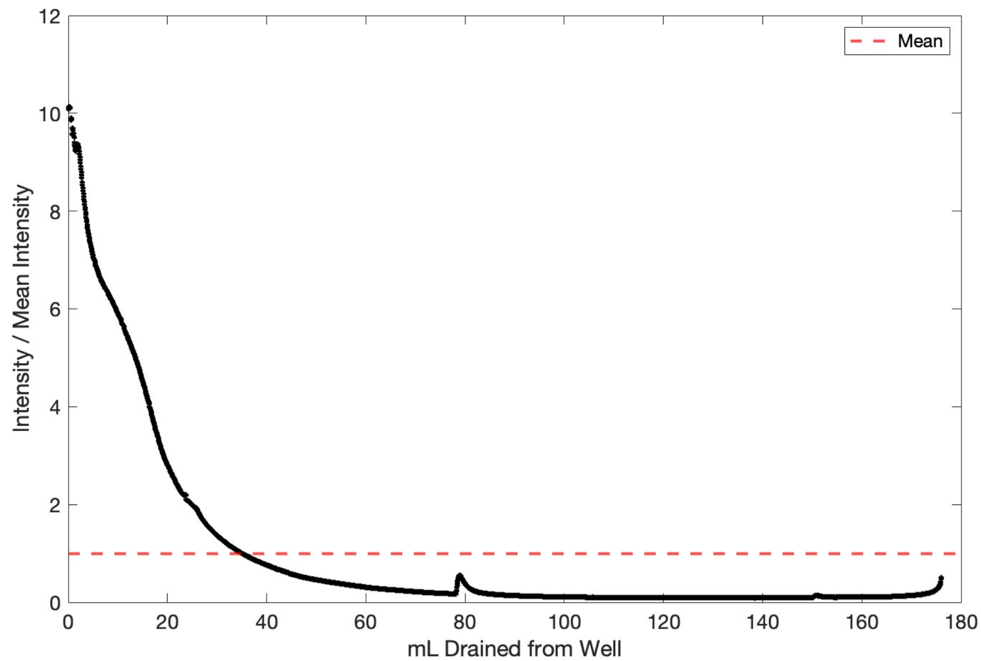

Figure 29: Jet Nozzle, Coefficient of Variation = 196.27%

The results of this test were promising, with a CoV below that of the control nozzle. However, this still left quite a lot of room for improvement, with the fluid still being far from homogeneous. The lower CoV was likely due to the slight decrease in outlet area, which caused a slight increase in fluid velocity at the outlet. This marginally increased mixing.

Shower Head Nozzle

The shower head nozzle was the second custom nozzle to be tested. This nozzle was designed to aspirate and dispense fluid from four different holes, as well as utilize an internal mixing chamber, as outlined in Chapter 3. The results of the shower head nozzle test are illustrated below in **Figure 30**.

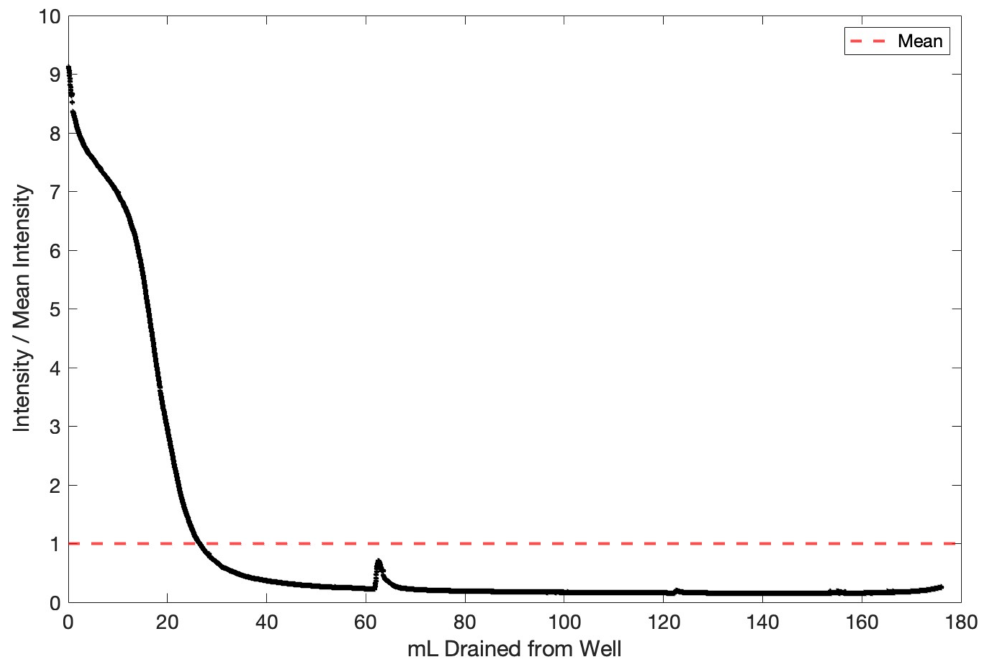

Figure 30: Shower Head Nozzle, Coefficient of Variation = 205.67%

With a CoV of 205.67%, this nozzle performed better than the control, but it did not result in better performance than the more basic jet nozzle. This is likely due to the four holes at the bottom of the nozzle. Although this resulted in a wider area of dispensation, the increase in total outlet area decreased the fluid velocity, which slowed the mixing.

Propeller Balls

Finally, tests were performed on the propeller balls designed to sit inside the well. The test consisted of four dense propeller balls which sank to the bottom of the well. These were placed directly adjacent to the outlet nozzle, which would, in theory, promote the agitation of the propeller balls, and therefore, surrounding fluid. The results of this test is displayed in **Figure 31** below.

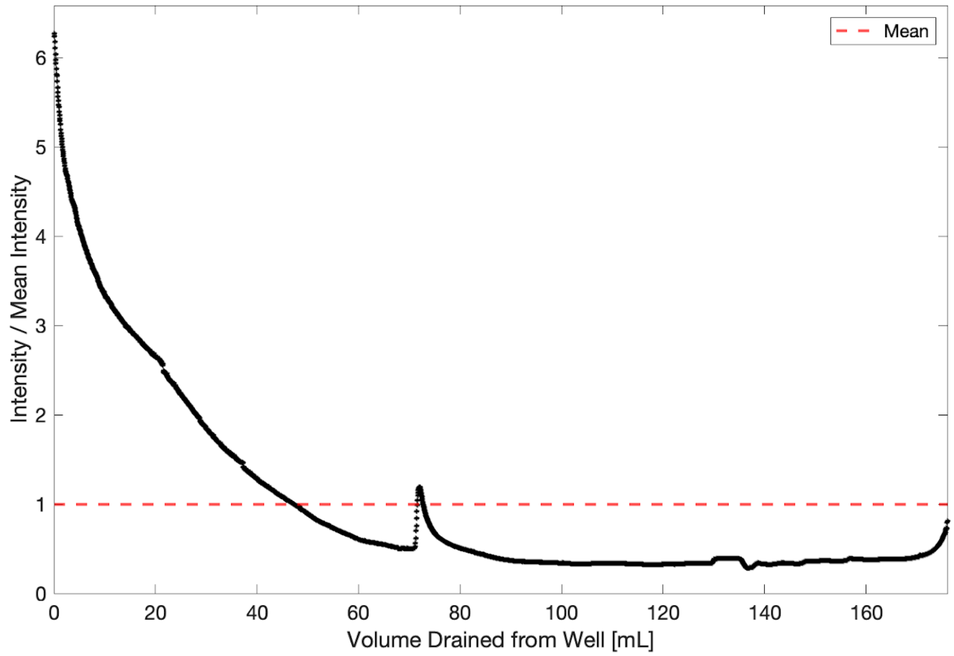

Figure 31: Four 10 mm Propeller Balls, Coefficient of Variation = 220.27%

This test yielded quite poor results, almost exactly equal to that of the control. However, this was expected once the team observed that the fluid flow from the outlet was unable to agitate the propeller balls at all; they didn't move throughout the entirety of the testing. This is due to the fact that, when lying on the bottom of the well, the static friction between the balls and the well's surface resists any attempt to spin the balls. Because of this, and the low flow rates of mixing, these propeller balls were unable to spin as designed. Because of this, additional testing of larger propeller balls was suspended, and other, more promising designs were focused on.

Check Valve Apparatus

The check valve apparatus was another important test, since this design did not focus on nozzle geometry; rather, it focused on altering where the fluid was dispensed compared to where it is aspirated, without complex, programmed mechanical movement. After the first test, this design was chosen as the final mixing strategy to be delivered to Illumina. Because of this, the check valve apparatus was tested a total of three times to ensure accuracy. The test results for this design are illustrated in **Figures 32a-c** below.

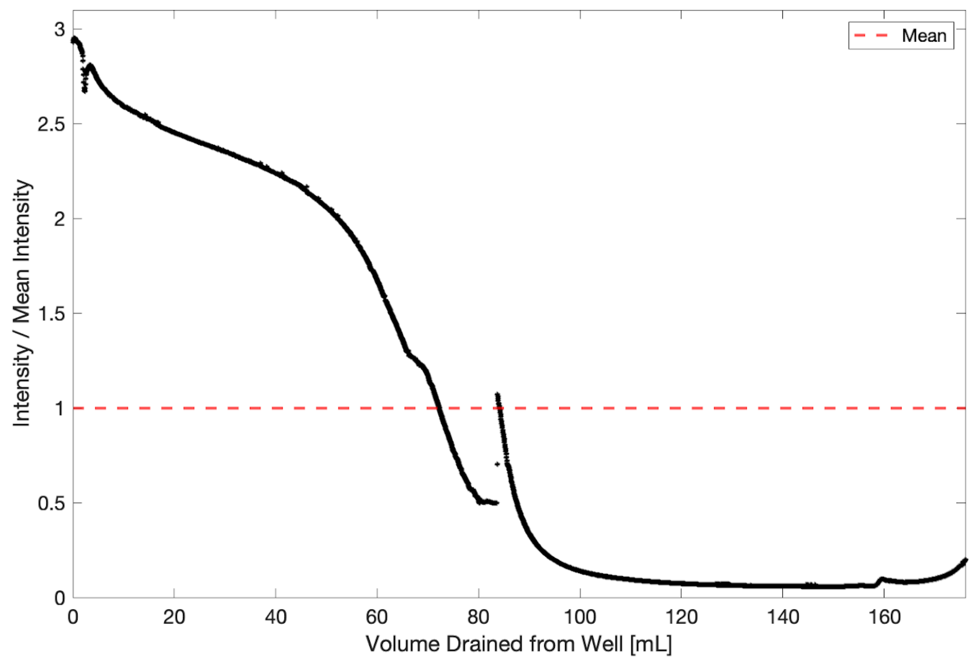

Figure 32a: Check Valve Apparatus, Trial 1, Coefficient of Variation = 103.02%

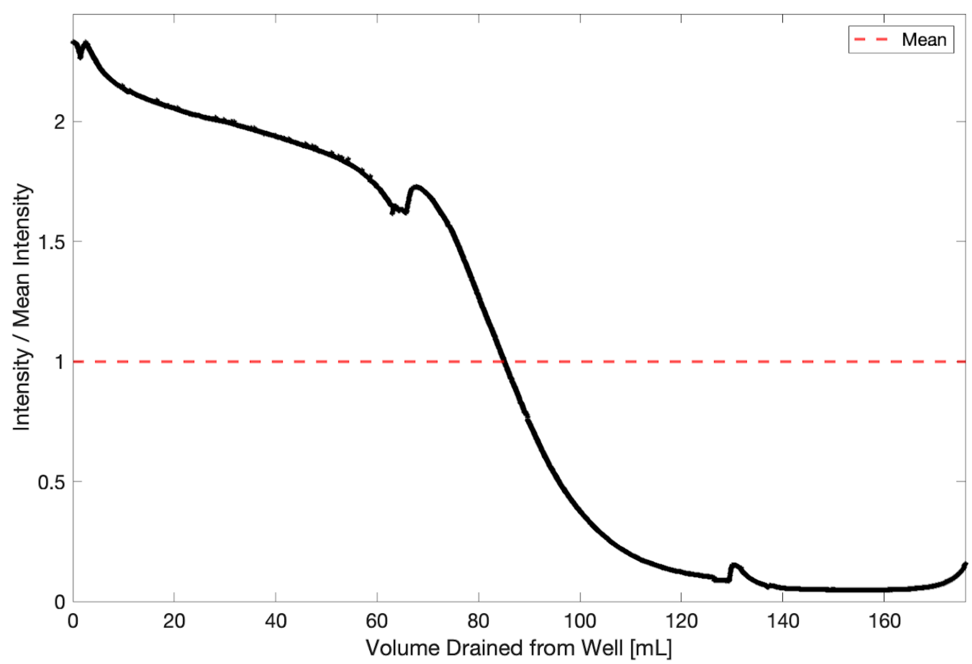

Figure 32b: Check Valve Apparatus, Trial 2, Coefficient of Variation = 86.75%

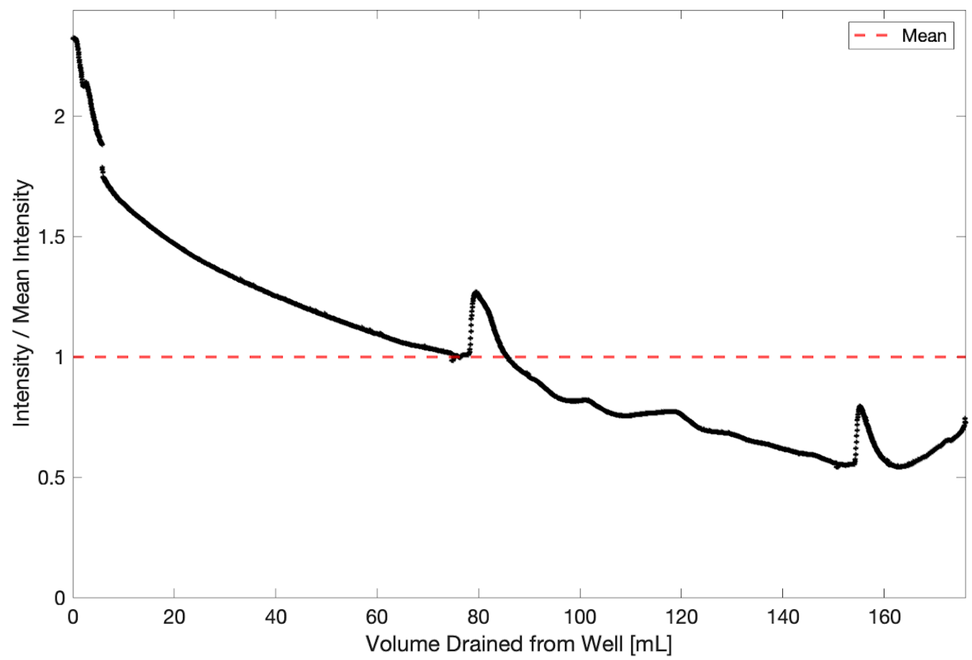

Figure 32c: Check Valve Apparatus, Trial 3, Coefficient of Variation = 37.47%

These tests resulted in an average CoV of 75.75%, the highest performing out of any design. For this reason, the check valve apparatus was chosen as the final deliverable. This performed in line with what the team expected; this excellent performance is due to the reagent being redistributed to the top layer of the well, as opposed to sitting at the bottom.

T-Nozzle

The T-nozzle, although originally intended for use with the check valve apparatus, was also tested as a standalone nozzle. This was tested the same as every nozzle, placed at the bottom of the well.

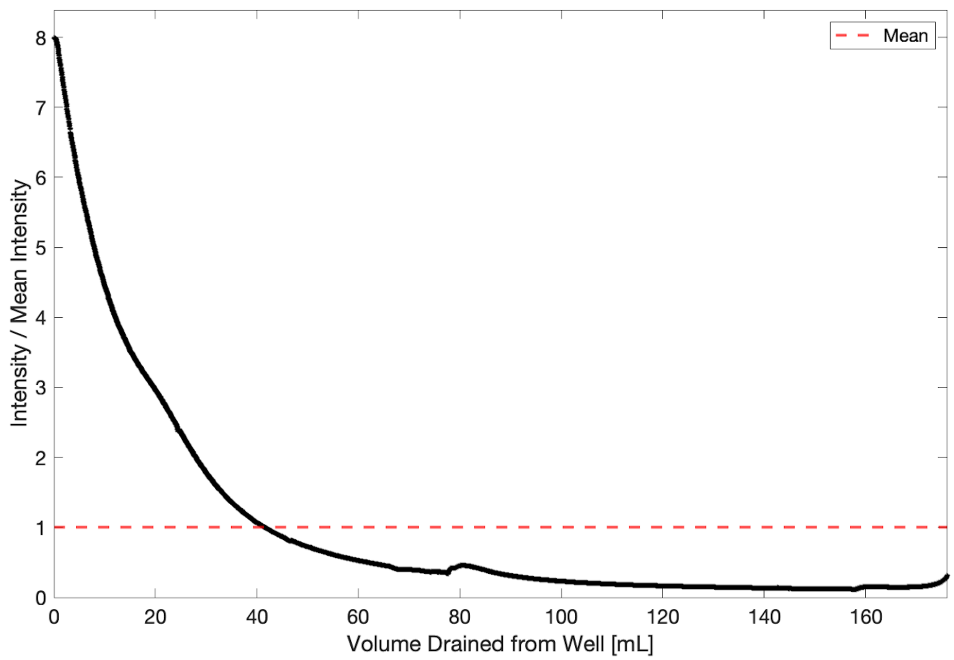

Figure 33: T-Nozzle, Coefficient of Variation = 156.64%

With a CoV of 156.64%, this nozzle yielded much better performance in comparison to other one piece nozzle designs such as the jet nozzle or shower head nozzle. As opposed to typical downwards facing nozzles, the sideways outlets on the T-nozzle allowed for further dispersion across the well, even propelling some reagent up the side.

Check Valve Apparatus With T-Nozzle

Additionally, the T-nozzle was utilized as an alternate dispensing nozzle for the check valve apparatus. This way, instead of dispensing downwards, it would dispense the fluid sideways near the top of the well. Because this was an offshoot of the final design, three tests were performed, as shown below in **Figures 34a-c**.

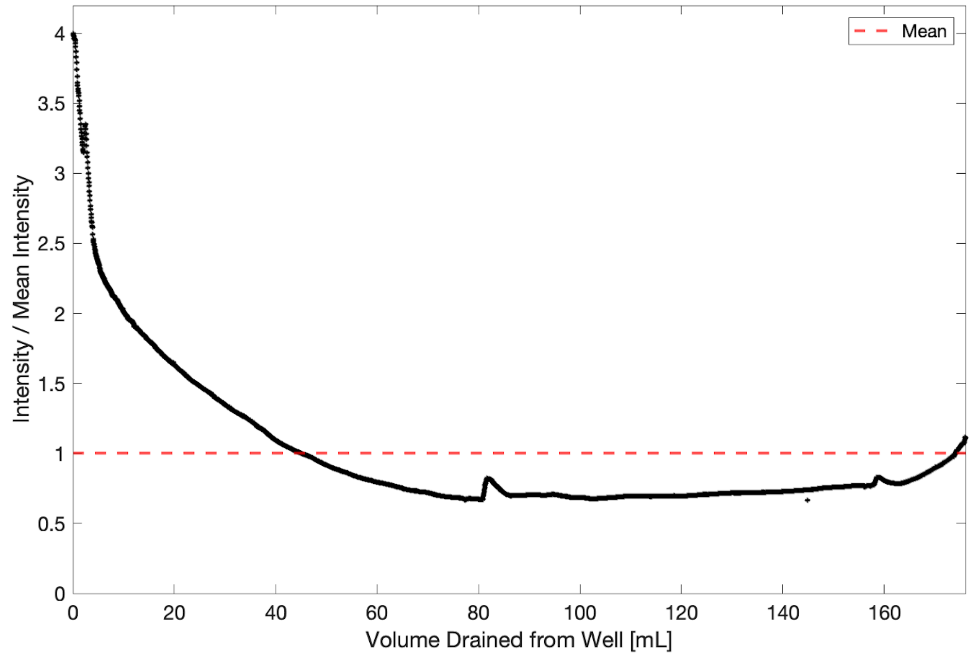

Figure 34a: Check Valve Apparatus with T-Nozzle, Trial 1, Coefficient of Variation = 52.63%

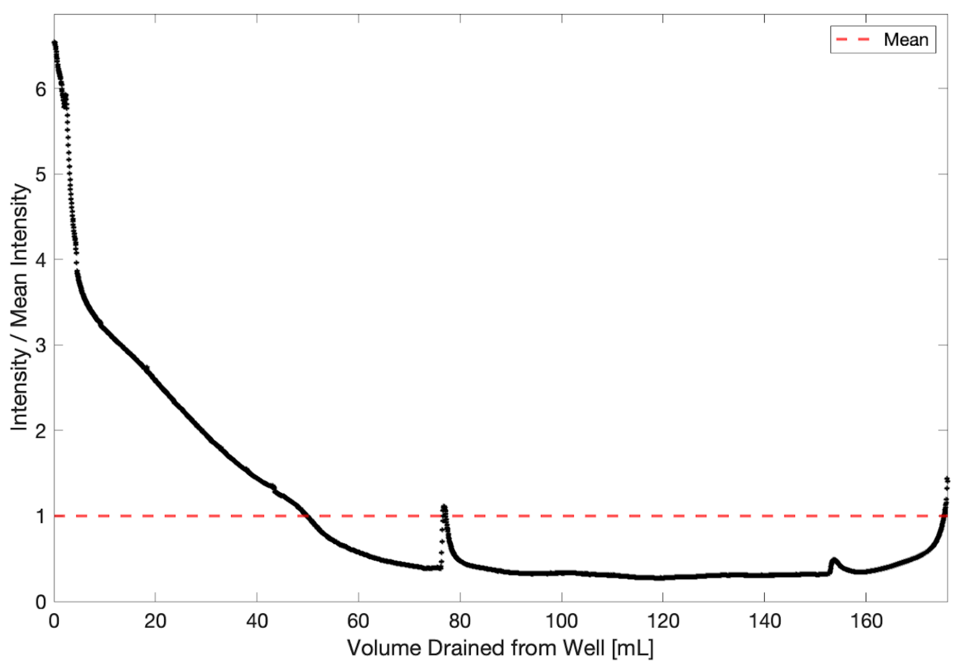

Figure 34b: Check Valve Apparatus with T-Nozzle, Trial 2, Coefficient of Variation = 114.62%

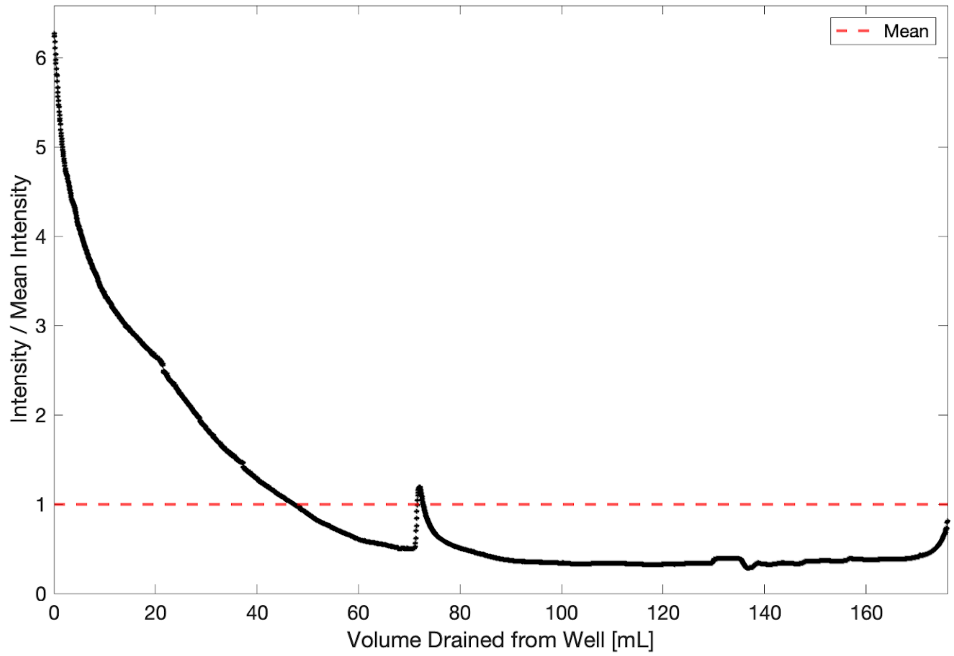

Figure 34c: Check Valve Apparatus with T-Nozzle, Trial 3, Coefficient of Variation = 109.98%

Surprisingly, this change caused the check valve apparatus to perform worse, with an average CoV of 92.41%, as opposed to the original check valve apparatus (CoV = 75.75%). This may be due to the fact that the first dispersion of the reagent into the well goes straight to the sides of the well, not the bottom. Because of this, the aspirating nozzle receives buffer instead of reagent, which is then redistributed at the top, pushing the buffer downwards again. By the time the reagent resettled at the bottom, the mixing process was over. Overall, this design was very interesting, and should be experimented with further.

Chapter 5: Design Recommendations and Conclusions

Design Recommendations and Conclusions

The check valve apparatus was the clear winner between the several designs tested. However, every design tested provided valuable insight on the issue of mixing, and there are several core ideas to be taken away from the project:

- The reagent tends to settle towards the bottom of the well. Designs that can combat this tendency by moving it upwards, as the check valve apparatus does, are generally successful.
- Increased fluid velocity improves mixing. Whenever possible, increase the fluid velocity at the outlet of the nozzle. Of course, this is subject to limitations, such as bubble formation and the prevention of high pressure differences within the system. Fluid velocity can be increased by:
    - Increasing flow rate
    - Decreasing cross sectional area of the outlet
- When using a check valve dependent design, use check valves with low cracking pressures. The cracking pressure is the minimum pressure needed to allow fluid flow, and a cracking pressure that is too high can prevent function of the system, stopping fluid flow entirely.
- Due to the pressure needed to activate a check valve, it is also important to minimize air in the system. Compressible air can end up delaying the transfer of pressure throughout the system, as opposed to an incompressible fluid such as water.
- Due to the importance of these pressure differences to a check valve dependent design, it would be beneficial to implement a pressure monitor into the test bed.
- Generally, when dispensing, it is beneficial to place the nozzle as high as possible.
- Likewise, when aspirating, place the nozzle as low as possible.
- If possible, increase the internal diameter of the fluid line when possible. This decreases fluidic impedance, which both increases the efficiency of the system and is quicker at transferring pressure differences.

Applicable Standards

In all disciplines in engineering, there is an applicable set of standards one must follow in order to produce reliable, safe, and notable results. One such standard followed heavily in this project was the ASME Y14.5 standard for

Geometric Dimensioning and Tolerancing (GD&T), which is used in every single component drawing in this report.

Additionally, when working with chemicals, such as fluorescein, BSA, and polysorbate 20, Material Safety Data Sheets (MSDS) were researched, studied, and followed in order to safely handle the relevant materials.

Impact on Society

The conclusions drawn by this project will aid in reducing the amount of time for not only Illumina, but every research group that is conducting genome sequencing using Illumina's testing kit. Renovating the current nozzle used by Illumina will not only reduce the carbon footprint of this process to make a safer environment, but also reduce both the manufacturing cost and time of Illumina's kit and costs to conduct genome sequencing for other groups. By optimizing this mixing strategy, genome sequencing itself will become more affordable and efficient, as Illumina will be able to fully implement lyophilizing methods for shipping and reduce shipping cost. A reduced cost will welcome more researchers to participate in, and therefore, help facilitate the research in relevant fields such as viral sequencing for COVID-19 virus and more.

Professional Responsibility

The global, economic, environmental and social responsibilities of this project align similarly to those of Illumina. Their primary goal was to be able to sequence entire genomes as fast as possible while minimizing error. The most optimal mixing strategy should be very accurate and reliable in helping detect various diseases.

The engineers on the team had a responsibility of conducting their work with ethics in mind. This includes the possible impact on society, as outlined previously, as well as proper reporting of results to the sponsor, whether good or bad. All data recorded was properly delivered to the sponsors of the project, with all relevant findings attached. All possible viewpoints of the project were considered, including how the results could affect Illumina and society as a whole.

Additionally, all references and sources of knowledge were properly cited and acknowledged, as seen in the Acknowledgements and References sections.

Acknowledgements

As the primary sponsors of this project, Norman Khoo and Stephanie Ogrey have been great guides and sources of plentiful knowledge. They have helped connect the team to various workers at Illumina and provided their time, effort, and knowledge to help advance the project in creating a better mixing strategy to improve the genome sequencing industry.

Brian Archer, a Fluidics System Engineer at Illumina, also volunteered to guide this project. He has provided the team with specific feedback for each design, tools to run the experiments, and endless knowledge on the methods and processes needed to conduct effective experiments. His help was absolutely invaluable.

Jerry Tustaniwskyj, the instructor of the team's MAE 156B section, was a consistent help in reviewing the team's work, critiquing design decisions, and assisting in project management.

Tom Chalfant, a Senior Machine Shop Manager at MAE department of UCSD, provided expert help in fabricating the complex designs of nozzles such as Showerhead and Jet nozzle.

References

[1] Wang, Yunfei, et al. “Needle-Free Jet Injectors' Geometry Design and Drug Diffusion Process Analysis.” Applied Bionics and Biomechanics, U.S. National Library of Medicine, 8 Nov. 2021, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8592715/

Appendix A: Project Management

Task Distribution

Bryson Pierce

Led development of test bed, developed data acquisition and analysis processes. Assisted in the conceptualization and/or design of prototypes, including check valve apparatus, T-nozzle, and propeller balls.

- Role: Fiscal manager
    - Managed budget and submitted purchase requests for the team.
- Conceptualized:
    - Post-mixing aspiration nozzle
    - Check valve apparatus nozzle
- Designed:
    - Check valve apparatus
    - Propeller balls
- Designed, and manufactured:
    - Camera mount
    - Tubing mount
- Managed development of test bed
- Created mixing recipes
- Programmed code for data analysis
- Created testing procedures
- Conducted all successful prototype tests
- Outlined, created, and consistently updated Gantt chart

Chen (Derek) Liu

Member of the design team. Proposed and designed multiple prototypes, including Shower Head Nozzle, Jet Nozzle, and Check Valve Apparatus. Collaborated on prototype manufacturing and test bed development.

- Role: Webpage and Documentation manager
    - Managed documents and designed the team webpage
- Conceptualized:
    - Shower Head Nozzle
    - Jet Nozzle
    - Tesla Valve
    - Check Valve Apparatus
- Designed:
    - Shower Head Nozzle
    - Check Valve Apparatus
    - Post-Mixing Aspiration Nozzle
- Collaborated Designed:
    - Jet Nozzle
    - Propeller Balls

    - Check Valve Apparatus Nozzle (T- Nozzle)
- Manufactured and Collaborated Manufactured:
    - Post-Mixing Aspiration Nozzle
    - Shower Head Nozzle
    - Jet Nozzle
    - Propeller Balls
    - Check Valve Apparatus and Nozzle (T- Nozzle)
- Helped conducted majority of prototype tests
- Conducted CFD analysis on the nozzles

Diego Padilla

Member of the sub team for designing. Proposed and helped design several prototypes, including fluidic slide. Collaborated with manufacturing Check Valve Apparatus, and test bed development.

- Role : Webpage and Documentation manager
    - Managed documentation and photos
- Conceptualized :
    - Fluidic Slide
    - Check Valve Apparatus
- Designed :
    - Fluidic Slide
- Created numerous figures and took documented pictures of final designs
- Extensive revisions so final report and poster
- Helped conduct several prototype tests such as jet nozzle, shower head nozzle, check valve apparatus, and T-nozzle.

Josie Han

Member of test bed, data acquisition, and analysis. Assisted in 3-D printing prototypes of propeller balls, camera stand, and getting resources for the lab

- Role: Sponsor Liason
    - Arranged meeting and presentation times with sponsor and communicated problems
- Assisted in test bed development
- Designed and Manufactured camera stand
- Mixed together buffer and reagent solutions
    - found proper places to store the reagents
- Assisted in testing processes
    - Helped conduct several prototype tests such as jet nozzle, shower head nozzle, check valve apparatus, and T-nozzle.
- Made ASME technical drawings for different designs that helped with GD&T
- Worked with instructors to get a private labspace for test bed set up

Syed Rizvi

Led design and manufacturing team by ensuring parts are designed for manufacturability (DFM) including Showerhead Nozzle, Jet Nozzle, Tesla Valve, and Check Valves Apparatus (CVA). Collaborated on full scale testing for multiple nozzle designs.

- Role: Safety Manager
  - Insured all leak-free and rigid connection for all fittings on test bed
- CAD and conceptualized:
  - Jet Nozzle
  - T-Nozzle
  - Showerhead Nozzle
  - Nozzle Hub
  - Check Valve Apparatus (CVA)
  - Nozzle Clamps for CVA
- Fabrication: (GD&T, Material Selection, 3D Printing and CNC)
  - Jet Nozzle
  - Showerhead Nozzle
  - T-Nozzle
  - Nozzle Hub
  - Tesla Valve
  - Nozzle Clamps for CVA
- Contributed in conceptualizing camera mounts
- GD&T including ASME Technical Drawings
- Reviewed testing procedures for hazard-free testing
- Collaborated in full scale testing

Risk Reduction Effort

At the beginning of the project, the team had to identify a high risk area of the first phase of the prototyping. It was decided that the primary test bed equipment – the valve and syringe pump – was a particularly important and complex area. For this reason, the objective of the risk reduction was to use these two pieces of equipment to successfully and automatically mix two different fluid reservoirs. Overall, this included:

- Operating the valve and syringe pump via computer software
- Creating an automated mixing “recipe” that mixed two fluid reservoirs
- Understanding fluidic fittings and how to properly install them

This was accomplished, but not after much effort and many failures. This was a great choice as a Risk Reduction effort, as many issues had to be addressed before the rest of the project began.

Intermediate deadlines

To manage project logistics, including intermediate deadlines, individual responsibilities, and a general timeline, a Gantt chart was used. This Gantt chart can be found here.

Appendix B: Bill of Materials, List of Suppliers, and Budget

Bill of Materials

Item	Manufacturer	Part #	Quantity
Y Connector	IDEX	P-512	1
Check Valve, Outlet	IDEX	CV-3316	2
1/4"-28 Union	IDEX	P-603	1
1/16" OD Clear Tubing, 5 ft	IDEX	1528	1
1/4"-28 Nut, 1/16" OD Tubing	IDEX	P-287X	3
1/4"-28 Nut, ⅛" OD Tubing	IDEX	P-331	2
Ferrule, 1/16" OD Tubing	IDEX	M-650	3
Ferrule, ⅛" OD Tubing	IDEX	P-350	2

List of Suppliers

- McMaster-Carr
- IDEX
- Cole-Parmer
- Amazon
- Millipore Sigma

Budget

- $5,000 budget, $1420.66 used
- Link to itemized sheet

Appendix C: Technical Drawings

- Camera Mount
- Tubing Mount
- Aspirating Nozzle
- Nozzle Hub
- Shower Head Nozzle
- Jet/Pencil Nozzle
- Tesla Valve
- T-Nozzle
- Propeller Balls

Appendix D: Component Analyses

Individual Component Analysis

- Bryson Pierce: CFD Software
- Chen Liu: Well Design
- Diego Padilla: Floating Devices
- Josie Han: Camera and Data Acquisition
- Syed Rizvi: Nozzle Geometry

Appendix E: Equations/Calculations and Code

Equations and Formulas Used

- $Q = A \cdot v = A\frac{\Delta x}{\Delta t}$ **Equation 1**
- $\Delta t = A\frac{\Delta x}{Q}$ **Equation 2**
- $\delta = \frac{\sigma}{\mu}$ **Equation 3**
- $\%\ Increase\ in\ Efficiency = (1 + \frac{CoV_2 - CoV_1}{CoV_1})^{-1}$ **Equation 4**

Code and Raw Data Repository

- Github: Illumina-Fluid-Cartridge
  - Kept private for concerns of IP.