Engineering Design

Research Proposal

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Gauging the Effectiveness of Microfluidic Gradient Devices

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INTRODUCTION

Abstract

Microfluidic devices are generally defined as glass, silicon, or polymer bodies in which a series of micro scale channels are etched or molded. It is through these channels that liquids are injected and are forced into a specific behavior by the channel's geometry. These devices are often modular in design in order to allow for a range of analysis and processing systems within a compact area. Microfluidics have gained a growing value to the scientific community in recent years as various substances such as antibiotics or often expensive cancer treatments can be introduced into these channels at extremely small doses, thereby using less reagent, and travel through the channels to be applied to a single or multiple test subjects simultaneously[1]. Two of these modular systems are mixing devices and gradient devices; using specific channel configuration and geometry, QL/mL amounts of reagent can be mixed and distributed asymmetrically to provide varying concentrations. However, due to their highly intricate and primarily internal nature, microfluidic devices are inherently difficult as well as expensive to produce by conventional means. The work presented here examines the accuracy and precision of two types of 3D printing process with respect to microfluidic fabrication. Mixer and gradient microfluidic devices were fabricated using the Ultimaker 5S FDM (Fused Deposition Modeling) printer and the Formlabs Form 2 SLA (Stereolithography) printer. Using image analysis techniques, channel size and quality were assessed. Data suggests both printing techniques were comparable with a 6-7% error in channel size. While FDM devices were less expensive to produce than SLA devices, it appears the SLA technology may prove to provide better quality (and are more capable of producing) smaller channel sizes.

[1] Ward and Fan, "Mixing in Microfluidic Devices and Enhancement Methods."

Gradient Microfluidics

The primary goal of this research was to assess the effectiveness of Gradient Microfluidic Devices, however at this time it simply covers the comparison of printing techniques. Gradient Microfluidics feature the ability to asymmetrically mix substances introduced into the channels resulting in a range, or gradient of output mixtures. This is achieved by having the two substance's channels merge along a centralized point and then travel down a serpentine-like channel to enable mixing by diffusion. The process can then be repeated, with the number of output channels increasing at each stage, ultimately resulting in a 'range' or gradient of outputs.

3D printing Microfluidic devices

In recent years, researchers have begun to turn to 3D printing as a way to rapidly produce microfluidic devices. Developed in the early 1980's, 3D printing was a technology that only in recent years has become readily accessible to consumers and functions by converting Computer Assisted Design (CAD) models into physical objects. A journal written by researchers at the Cardiff School of Engineering explores the prospect of FDM printing these models, testing the effects of print orientation, layer height, print speed, and print material. The researchers found their models, printed at a layer height of .05mm-.025mm and a channel diameter of 1.4 mm, within 2.5% of the designed dimensions on average and their design also provided capabilities similar to that of legos, allowing for modular capabilities. Additionally it was observed that the clarity of the model, printed in transparent PETG and ABS, was directly influenced by layer height and print speed with slower speeds and smaller layers increasing the clarity of the models. However, researchers also observed an upper limit to this improvement with minimal benefits coming from printing slower than 30 mm/s and very few market grade FDM printers being able to print at layer heights smaller than .05mm. The group ultimately came to conclude that these devices, while not absent of issues such as leakage and opacity, are effective in their tasks and successfully provide a much wider market with access to the field of microfluidics[2]

Within the journal titled "Lab on a Chip", researchers discuss the benefits and costs to 3D printed Microfluidics. They begin by noting how 3D printing's rapid prototyping capabilities, allowed testers to take on a "fail fast and often" strategy in design and production. The researchers go on to explain that they utilized various printing methods in their tests including SLA or Stereolithography, FDM, and 2PP or two photon polymerization. The primary issue researchers noted, arose with resolution. While quality can reach extremely high levels in modern 3D printing, up to .025mm layer heights, it often cannot compare to a manufactured or injection molded product, and even those that come close have the potential to suffer from artifacts or issues in the surface quality of the channel. The journal ultimately comes to these conclusions: SLA printing is precise and doesn't require support material within the internal body (depending on orientation) but is limited to UV curable resins. 2PP can achieve the most precise level of detail but is severely limited in the area the device can produce an object within. Lastly, FDM allows for a much wider range of materials at the cost of noticeably lower resolution channels and at the high risk of irremovable artifacts [3].

Methods

<u>Design Process</u>

[2] Morgan et al., "Simple and Versatile 3D Printed Microfluidics Using Fused Filament Fabrication."
[3] Waheed et al., "3D Printed Microfluidic Devices."

The process of designing a 3D printable Microfluidic device involves three key pieces: the input, the channels, and the output. The models were all produced in Fusion 360 v2.0.12376. The tested models featured a luer lock connector as our input, as it allows for secure attachment of BSTEAN 3mL syringes.

In producing the channels, the first task is to create a body, typically rectangular, to cut away from. Fusion 360's pipe tool was used in creating the channels, having it cut extrude a cylinder along a pre-sketched path with a pipe diameter of 2.5mm. The output was a hole in the opposite end of the model through which samples could drain. An additional luer lock or other connector could be added to this point however to create a modular setup.

3D printing strategies

In printing the tested models we used Ultimaker Cura v4.9 alongside the Ultimaker 5S FDM printer. Settings were those of Cura's default .1mm layer height with changes to print speed (30mm/s instead of the default 70mm/s) to improve clarity of models as suggested by the journal *Simple and Versatile 3D Printed Microfluidics Using Fused Filament Fabrication* [4]. The models additionally utilized only 75% infill, with a rectilinear pattern (Referred to as 'Lines' in Cura Slicer), in our prints as it was the minimum necessary to support the top surface of the model without wasting material, and thus time. One potential point of error in our FDM channel accuracy arises from the use of no support structures in printing. This was chosen, as in our testing with PVA water soluble supports the density of support material was too thick to be washed away, and thus rendered the model unusable. The model was printed flat with the luer locks on the top most surface.

[4] Morgan et al., "Simple and Versatile 3D Printed Microfluidics Using Fused Filament Fabrication ((Extremely Useful) See Overcoming the Fabrication Barrier Subheader and Device Fabrication)."

The SLA prints were sliced in Formlab's PreForm v3.22.1 slicer and were printed on a Formlabs Form 2 SLA printer in Clear V4 resin. The settings for these prints were similar, .1mm layer height and only external supports, with a change to the infill, increased to 100%, as required for SLA prints. Once the model is removed from the build plate, the channels are flushed repeatedly with syringes of 99% isopropyl alcohol. The model is then placed in the Formlabs Form Wash station for ten minutes. The channels are then filled with water and placed in the Form Cure at 60 degrees for 30 minutes with a pause every five minutes to re-flush the channels with water. To maximize clarity, an additional thin layer of clear resin can be brushed on to the model and then placed into the form Cure for 1-2 hours. A full list of settings can be found below in Figure 1.

Ultimaker 5S settings
Layer Height: .1mm
Initial Layer Height: .2mm
Wall Line Count: 3
Top/Bottom Thickness: 1.0
Top Layers: 10
Bottom Layers: 10
Infill Density: 75%
Infill Pattern: Lines
Infill Layer Thickness: .1mm
Printing Temperature: 200
Bed Temperature: 60
Print Speed: 30 mm/s
Infill Speed: 30 mm/s
Top/Bottom Speed: 21 mm/s
Travel speed: 150 mm/s
Generate supports: Disabled
Filament: Ultimaker PLA Transparent
Formlabs Form 2 settings
Layer Height: .1mm
Resin: Clear V4

Fig. 1 Ultimaker 5S (upper) and Formlab's Form 2 (Lower) print settings

Methods of analysis

In analyzing the models the java based image analysis software ImageJ v1.8.0. was used alongside a process known as thresholding. In order to threshold an image it must first be converted to a 32 bit image and then it is recommended to increase the contrast by up to .3%. The user then establishes a range of grey values (Fig 2.1 A) that will be converted black with values outside of that range turning white (Fig 2.1 B). The result is a version of the image in which each layer is approximately visible, allowing for the outer edges of the channel to be established. A profile is then plotted from one channel edge to the other (Fig 2.2 B) in which, due to the dual color status of the image, results in only max or min values (Fig 2.2 A). In establishing scale, a micrometer was used alongside a 3D printed mount. This was done to allow for the micrometer to rest at the same depth as the model without altering the microscope magnification and thus scale.

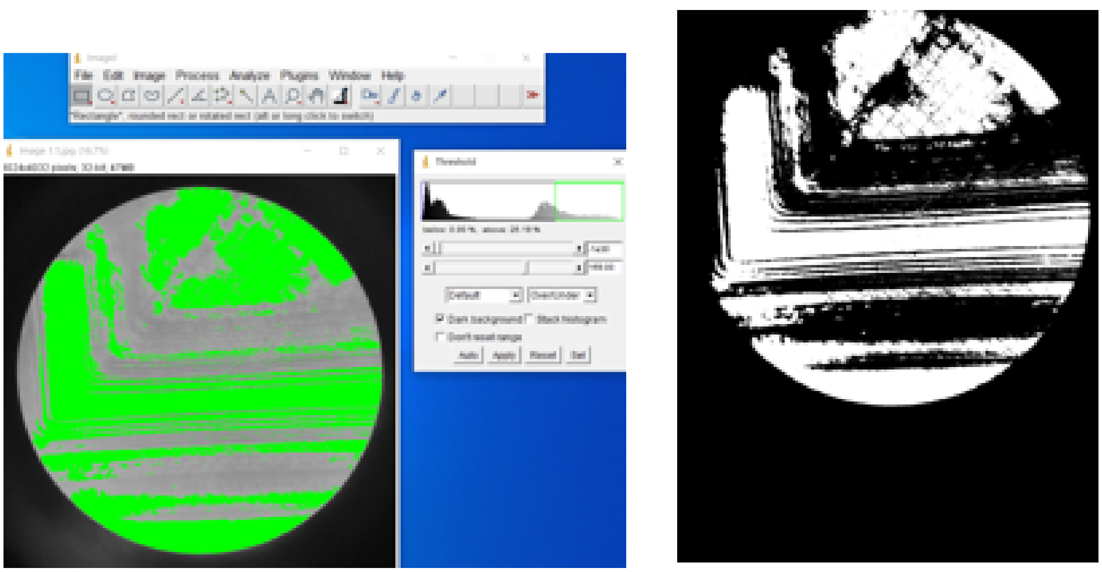
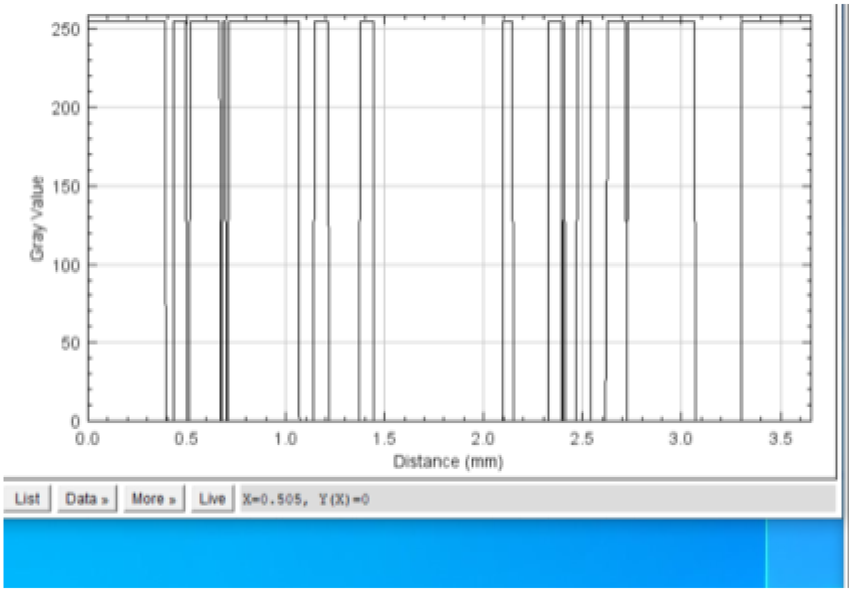


Fig. 2.1 A **Fig. 2.1 B**

Figure 2.1 Visualization of the thresholding process (A) with corresponding outputted image (B).



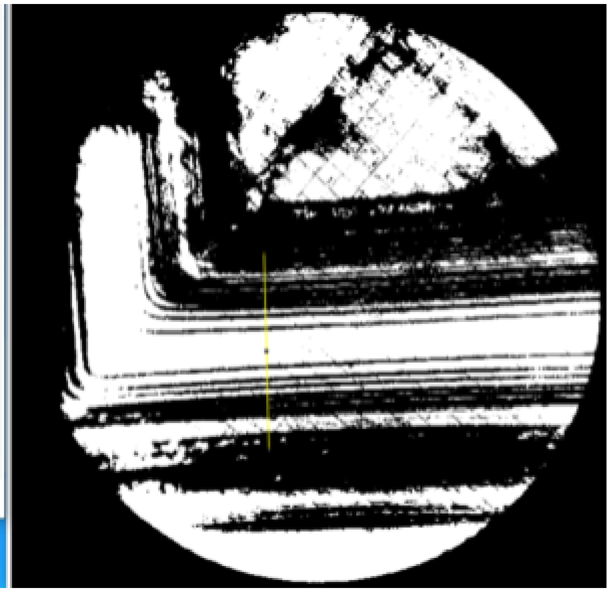


Fig. 2.2 A

Fig. 2.2 B

Figure 2.2 The plotted profile (A) of the thresholded image (B). Peak values represent the black regions of the image, typically at the edge of layers, whereas zeros represent white regions, typically seen as the flat area of a layer. The longer plateaus at the far edges represent the outer edges of the channel.

Gradient Verification / Future Work

Quantitative measuring of results

In previous testing of 3D printed bodies, researchers chose to measure the success of the gradient devices through a visible shift in color thanks to dyes mixed in with the two substances. This verification technique, however, suffers accuracy flaws based on the fact that it requires the human eye to gauge the shift in color, providing little to no quantitative information and causing the tests to grow increasingly inaccurate with smaller degrees of color change.

Provided additional time, future work will include the investigation of alternate means of fluid analysis and the gathering of quantitative information as to the effectiveness of a gradient device. One potential method of performing this analysis uses a color densitometer. A color densitometer is a device that utilizes a light source and a photocell to measure the density of primary colors present in a sample; this is achieved by measuring what wavelengths of light do and do not pass through the sample [5]. This device would be useful in measuring the precise differences in color, or dye, presence in samples and thus the effectiveness of the gradient device overall. This method requires individual samples from each gradient reservoir and must be able to be placed onto a clear microscope slide; thus requiring alterations to the model's output in future tests.

Another potential method as detailed in testing performed by researchers under MDPI, is data collection from a salinity gradient by utilizing a Schlieren imaging device. These devices, through the use of a parabolic mirror, a knife's edge (typically a razor blade), and a camera, allow researchers to see variances in air or other transparent media. Researchers from MDPI noted that these devices allowed them to detect density differences

[5] Phillips, "What Is a Colorimeter, and How Does It Work?"

in the salinity gradients and utilized that in gauging the success of the gradient [6]. The only changes to testing procedure in the use of this verification method is the mixing of salt into one of the two samples rather than dye in both.

A future requirement for all potential verification techniques is a method through which both substances can be injected into the machine at precisely simultaneously and at the same volume. Research is still being performed as to the best method for achieving this goal.

Verification Construction

In using the color densitometer for tests a manner in which the samples can be removed once mixed is necessary. This is both due to the densitometer not being able to fit the micro-fluid body as well as the need to isolate each test sample in measuring it. This may be achieved by taking an existing gradient design, and altering it to output into a series of tubes through which the samples may travel to isolated collection points. (Fig. 3.1).

[6] Sun, Chen, and Hsiao, "Mapping the Salinity Gradient in a Microfluidic Device with Schlieren Imaging."

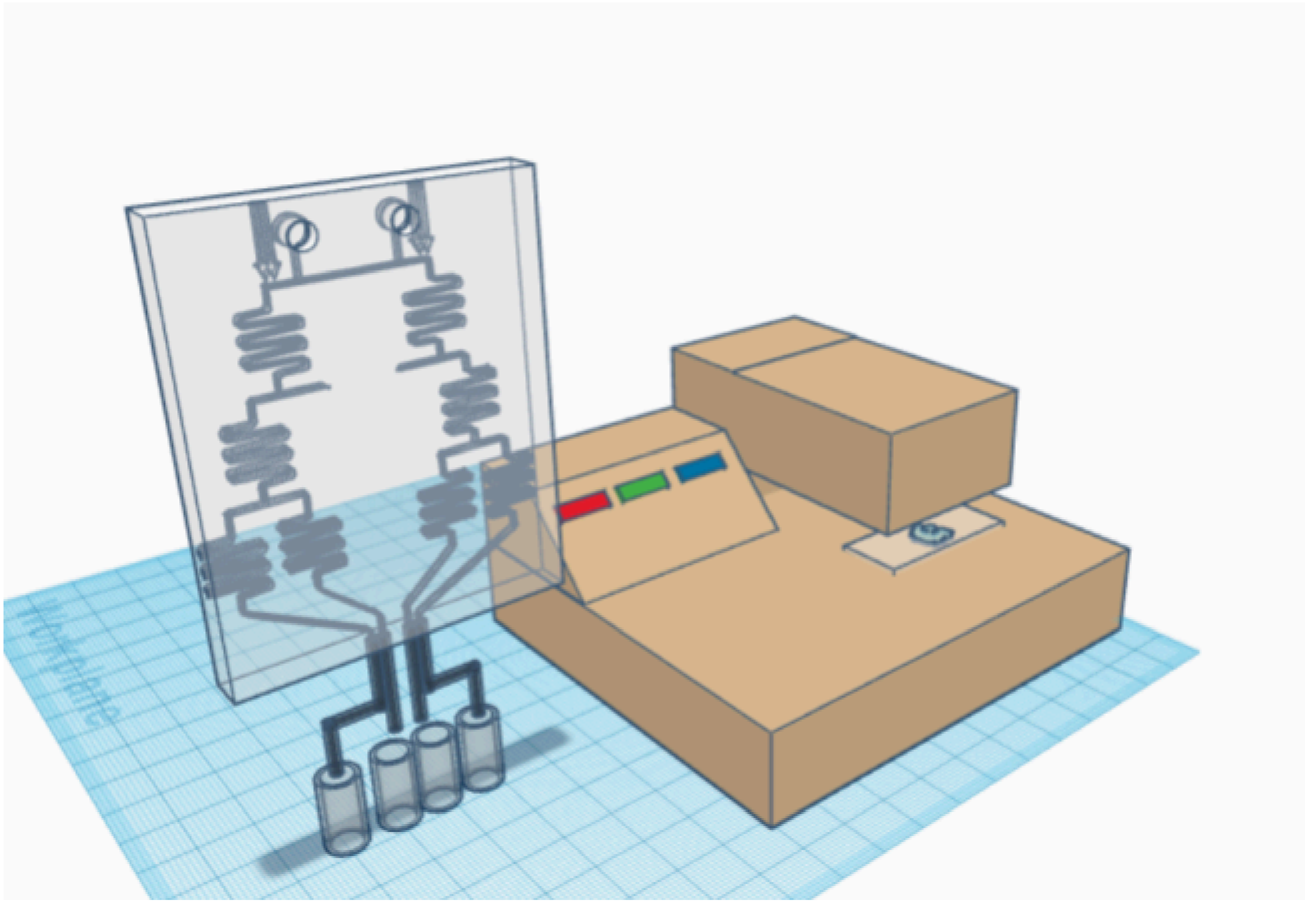


Figure 2. A model of a gradient microfluidic device with additional sample extraction outputs (Left) alongside a color Densitometer (right).

In order to use the Schlieren imaging system the device itself must first be constructed; consisting of a light source (a white LED will suffice), a camera, a spherical concave mirror with listed focal length (utilized in calculating distance from the camera), a mount for said mirror that rests two times the distance of the mirror's focal length from the camera, and a standard razor blade. The testing process requires that the LED shine directly on the mirror and that the reflection of the light passes through the sample. (Fig. 3.2) From there the camera can be positioned to look through a hole along the razor blade's edge.; the camera's exposure may need to be altered for best results. From the information gathered by these tests one can measure approximate percent salinity within each sample and compare it to what the predicted results of the gradient design would be.

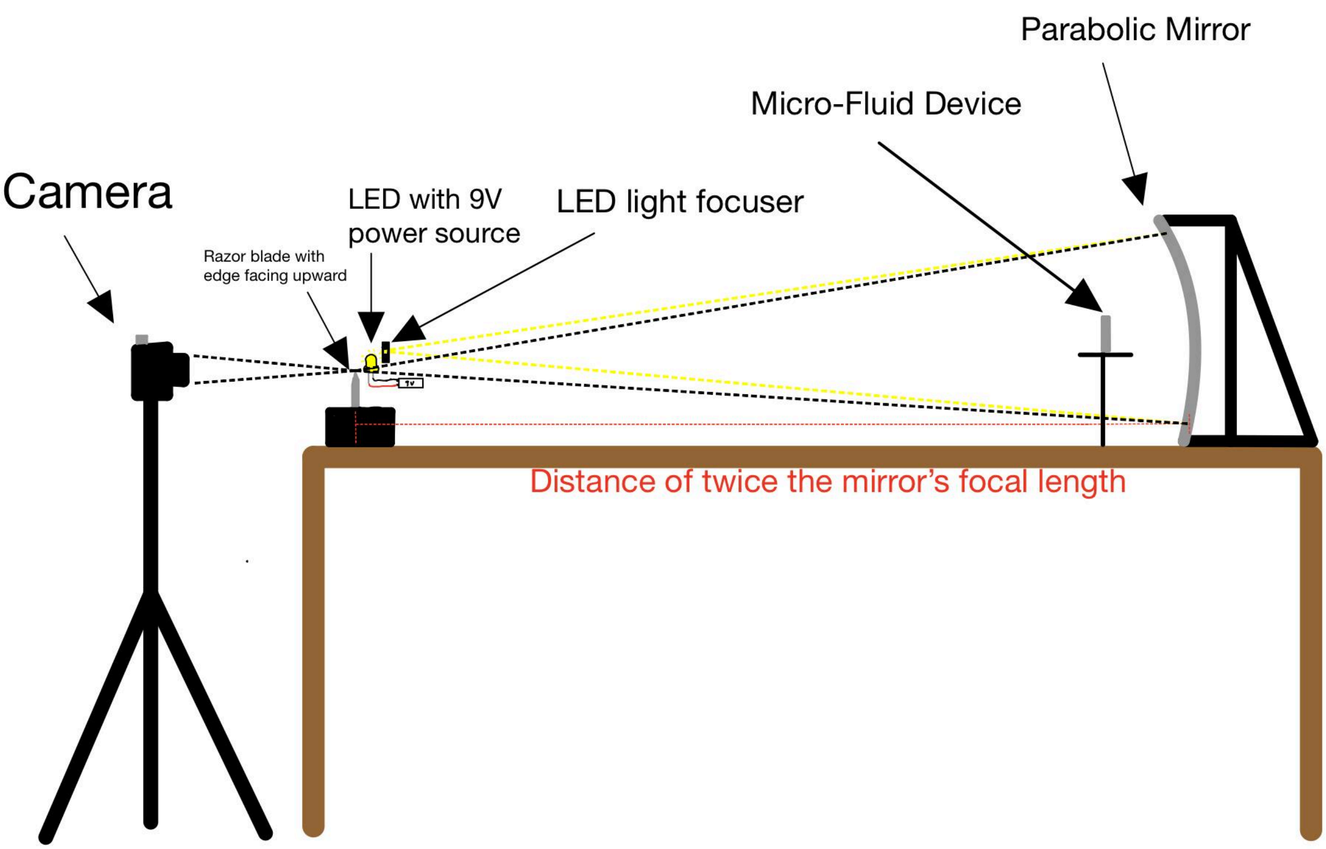


Figure 3. A diagram of a Schlieren imaging setup

Results

Due to time constraints, only a small amount of data was collected for these tests and the above verification models were not able to be constructed. The data taken from the Ultimaker 5S (Fig. 4.1) and Form 2 (Fig. 4.2) are listed below. Both the FDM and SLA devices were able to be successfully and repeatedly produced with an average error of only 6-7%. The trend of this data suggests that while FDM printing may require less post processing and be lower cost, future work should be conducted with SLA machines due to their precision and capability to improve quality beyond what was achieved in these tests.

	Left Edge	Right Edge	Distance	Percent Error*
Channel 1.1	0.32	3.29	2.97	18.80%
Channel 1.2	0.09	2.96	2.87	14.64%
Channel 1.3	0.16	2.14	1.98	20.77%
Channel 2.1	0.63	3.21	2.57	2.95%
Channel 2.2	0.46	3.36	2.90	15.93%
Channel 2.3	0.32	3.17	2.85	13.90%
Channel 3.1	0.24	3.03	2.79	11.66%
Channel 3.2	0.04	2.57	2.53	1.10%
Channel 3.3	0.06	2.61	2.56	2.21%
Average			2.67	6.71%
Std. Dev.			0.31	

Figure 4.1 Data acquired from the FDM models printed on the Ultimaker 5S.

	Left Edge	Right Edge	Distance	Percent Error*
Channel 1.1	0.06	2.37	2.31	7.51%
Channel 1.2	0.01	2.64	2.63	5.24%
Channel 1.3	0.02	2.36	2.34	6.24%
Channel 2.1	0.01	2.45	2.44	2.41%
Channel 2.2	0.05	2.46	2.41	3.51%
Channel 2.3	0.01	2.27	2.26	9.52%
Channel 3.1	0.03	2.71	2.69	7.43%
Channel 3.2	0.05	2.77	2.72	8.79%
Channel 3.3	0.02	2.60	2.58	3.33%
Average			2.49	6.00%
Std. Dev.			0.17	

Figure 4.1 Data acquired from the SLA models print on the Formlabs Form 2

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