Final Report

TABLE OF CONTENT

Project Objectives

- Design of high-throughput TRACER platform that compatible with the 96 well-plate formats
- Stacking 6 layers of scaffold which have regions blocked off PMMA
- Characterization of various parts of the device (custom well plate, scaffolds, syringe holder, seeding device/pipeline)
- Expansion design on smaller (384 well plate) design for other studies (and find possible uses of the design)

Component/progress

• A: 3D printing of the 96-well PMMA disk

o Demonstrating Proof of Concept(G-CODE)

To demonstrate that an experiment 3D printing PMMA wells was possible, we used G-code (automatedly generated by MATLAB) to produce sheets of 96 circular wells with the same diameter and relative spacing as that of a standard bottomless 96-well used in cell culture. Additional parameters were chosen arbitrarily including 4mm diameter,4 rings per well, and 0.5mm spacing between the rings (**Table A1**).

Table A1 – Proof of Concept prints for determining feasibility of PMMA circular printing (File: G_CODE_4mm_4LAY.mlx ; GCODE_4mm4LAY.gcode)

Trial#	Description	Imaging
1	4mm diameter 4 layers 0.5mm spacing 3000 speed	
2		
3		

*Note: The PMMA used is the old one Jose made. The PMMA concentration is 17.5% weight/volume

- **Print Pattern Characterization/Result**

At the end of the week of May 13-17 we decided on which treatments we wanted to test on the printer and compiled an Excel data table (**Table A2**). Twenty-four treatments were outlined. The data table was used to then create G-code for 3x3 grids of wells for each of the treatments, separated into four sheets with six treatments per sheet (denoted **tA1-24**).

Table A2 – Excel data table(File GCODE_multitesting.gcode)

Treatment #	radius(mm)	# of Layers	Layer Spacing(mm)	Speed(mm/s)
1	3.25	2	0.25	3000
2	3.0			
3	3.25		0.5	
4	3.0			
5	3.25	3	0.25	
6	3.0			
7	3.25		0.5	
8	3.0			
9	3.25	4	0.25	
10	3.0			
11	3.25		0.5	
12	3.0			
13	3.25	2	0.25	1000
14	3.0			
15	3.25		0.5	
16	3.0			
17	3.25	3	0.25	
18	3.0			
19	3.25		0.5	
20	3.0			
21	3.25	4	0.25	
22	3.0			
23	3.25		0.5	
24	3.0			

We started with a 17.5% volume/volume formula for our PMMA (denoted **Formula A**). Later into the week of May 21-24 we began printing tA1-6 and tA13-18 but found Formula A to be insufficiently viscous and bleeding into other wells. We then switched to a 17.5% weight/weight formula based on the molar mass of the PMMA powder (**Figure A1**), denoted **Formula B**.

Figure A1 – PMMA Formula B

- 1.75mg of PMMA
- 1.0mg of methyl Blue
- 7.84mL of 100% Acetone

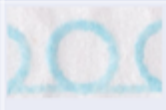
Instructions for production:

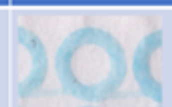
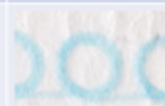
- Mass and combine all components, shake overnight
- Store in fridge for remainder of usage

First Round Trials

At the end of the week (May 24) we reprinted tA1-6 and tA13-18 with the Formula B, as well as printed tA7-12 and tA19-24 (**Figure A2**). With all the treatments printed with a uniform PMMA formula, we qualitatively identified **tA10** and **tA12** to have the best circular shapes with the easiest reproducibility. It was decided that we would now want to maximize and minimize the number of rings for tA10 and tA12, in order to view both extremes and choose the best final parameters.

Figure A2 – Prints for tA1-14 with PMMA formula B

Speed	D=6.5mm d=0.25mm 2LAY	D=6.0mm d=0.25mm 2LAY	D=6.5mm d=0.5mm 2LAY	D=6.0mm d=0.5mm 2LAY	D=6.5mm d=0.25mm 3LAY	D=6.0mm d=0.25mm 3LAY
3000						
1000						

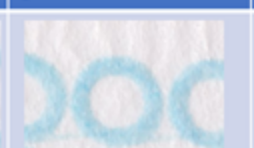
Speed	D=6.5mm d=0.5mm 3LAY	D=6.0mm d=0.5mm 3LAY	D=6.5mm d=0.25mm 3LAY	D=6.0mm d=0.25mm 3LAY	D=6.5mm d=0.5mm 4LAY	D=6.5mm d=0.5mm 4LAY
3000						
1000						

<u>Second Round Trials</u>
At the beginning of the week of May 27-31, we created G-code for tA10 and tA12, this time testing from a range of 4 rings to 9 rings around their wells, creating 3x3 grids for each number of rings (**Figure A3**). After this, we again visually identified which grid we preferred and chose our final parameters:

- 3.00mm inner radius
- 0.5mm ring spacing
- 4 rings per well
- 3000mm/ms printing speed

(These parameters are denoted as **tB1**)

Figure A3. tA10 and tA12 5-8 layers prints with PMMA formula B (File GCODE_6mm_5-8LAY.gcode ; GCODE_6mm_6LAY.mlx ; GCODE_6mm_8LAY.mlx)

Trial # / Layers	5	6	7	8
10 D=6mm d=0.25mm				
12 D=6mm d=0.5mm				

We printed a total of five 96-well sheets of tB1 (**Figure A4**), imaged them with a Canon PIXMA MX320 and analyzed the inner circle areas with ImageJ.

Figure A4. Final Print tB1 with PMMA formula B (GCODE_6mm_4LAY.mlx ; GCODE_6mm_4LAY.gcode)

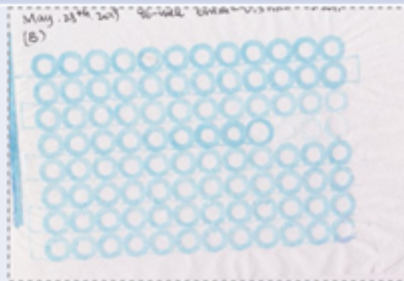
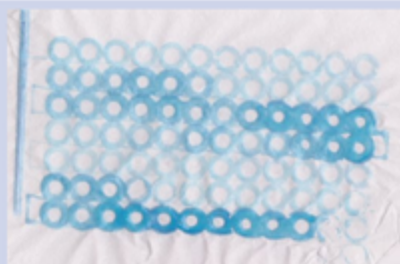
Trial #	Imaging
1	
2	
3	

Image Analysis

Each of the five tB1 prints was scanned (**Figure A5**). Two of the five were viable for image analysis producing the following measurements of area of the wells:

Figure A5. tB1 Imaged print

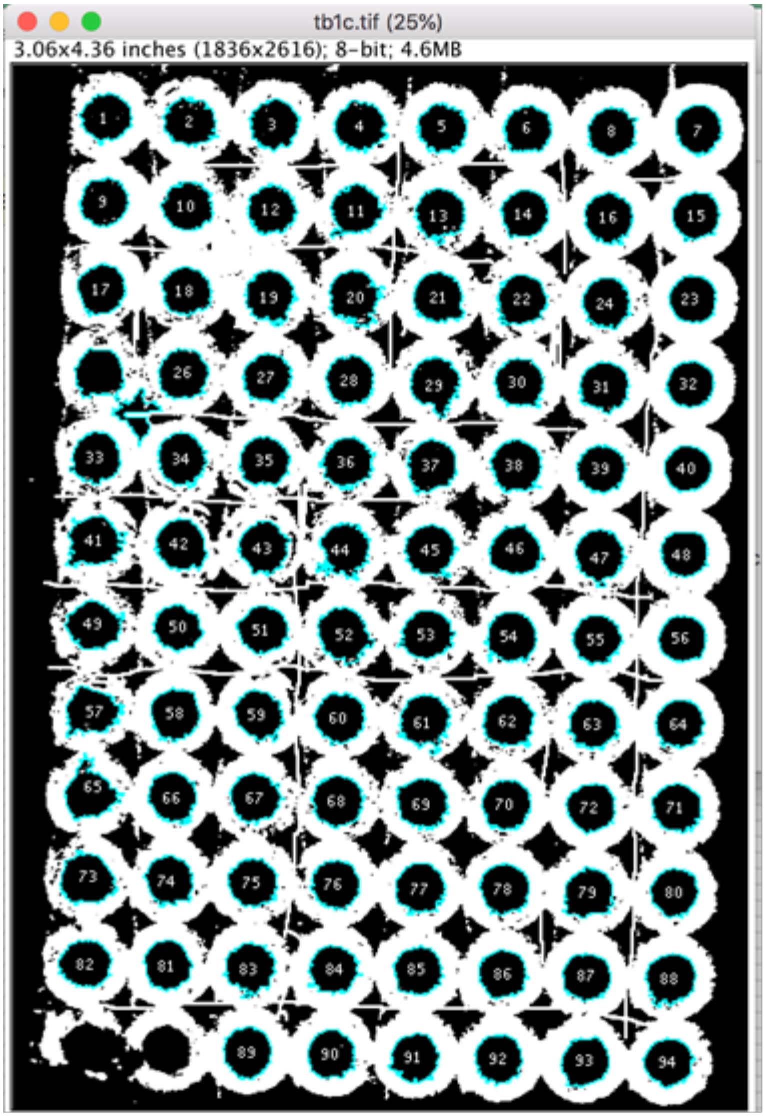
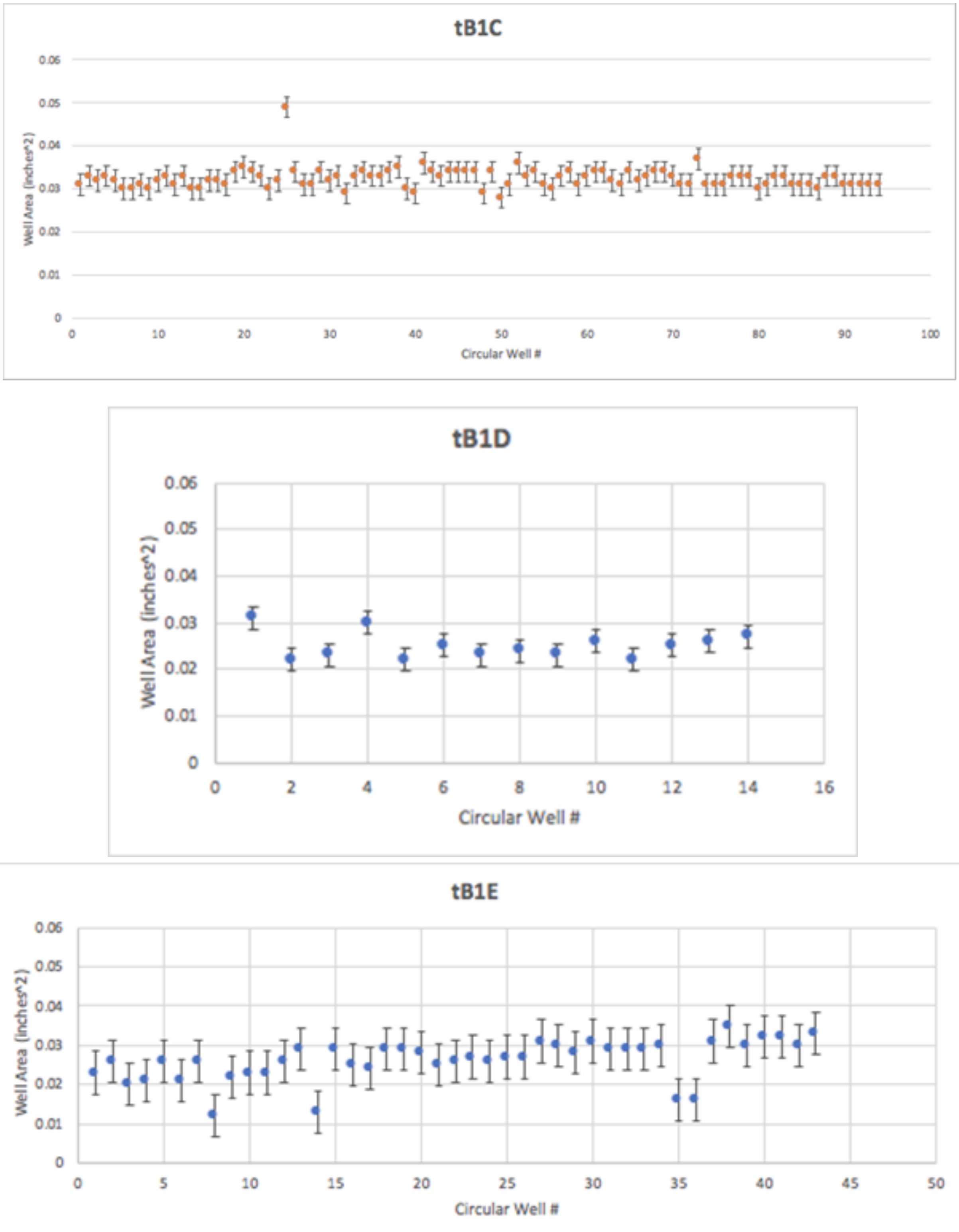


Table A3: Average well areas for 96-well PMMA-printed circles computed in inches^2.

Print	# of wells taken into account	Average well area (inches^2)
tB1c	94	0.03241489
tB1d	14	0.02492857
tB1e	43	0.02616279

Figure A6. Well Area Distribution for tB1C, tB1D, tB1E



We feel the average well area of the tB1c sheet is more reflective of the actual average well area value as all but two of its well areas were computed.

Discussion:

We encountered several issues over the course of the experiment thus far:

- ***Leakage of PMMA Formula A***

Formula A had leaked substantially into the paper (**Figure A7**) which prompted the switch to Formula B.

Figure A7 – tA1-6 print with Formula A (low viscosity), May22

- ***Presence of air bubbles in syringe tips***

Air bubbles in the syringe even after the pre-programmed linear brush strokes resulted in some 96-well sheets displaying incomplete circles. Mitigation of this issue requires slower movements when filling the syringe with PMMA and when aspirating.

- ***Damage of printing paper from adhesive tape***

During the printing process, the paper must remain completely flat on the platform and thus adhesive tape is commonly used to achieve this. However, tape sometimes damages the paper. An alternative solution would be preferred for keeping the paper intact, for example, designing clamps or using purchasing office clamps.

- ***Lowered Platform***

Due to the repeated pressures applied to the platform while taping/removing the paper, excessive applied force has caused the platform lower so that the tip is not touching the paper properly for the full duration of the experiment. Realigning the platform with its underlying screws is necessary for any further experimentation on the printer.

Tip selection/Characterization

With the issues surrounding inconsistent prints (see Report #1) we wished to investigate whether a different tip size would allow for a better flow of PMMA onto the paper.

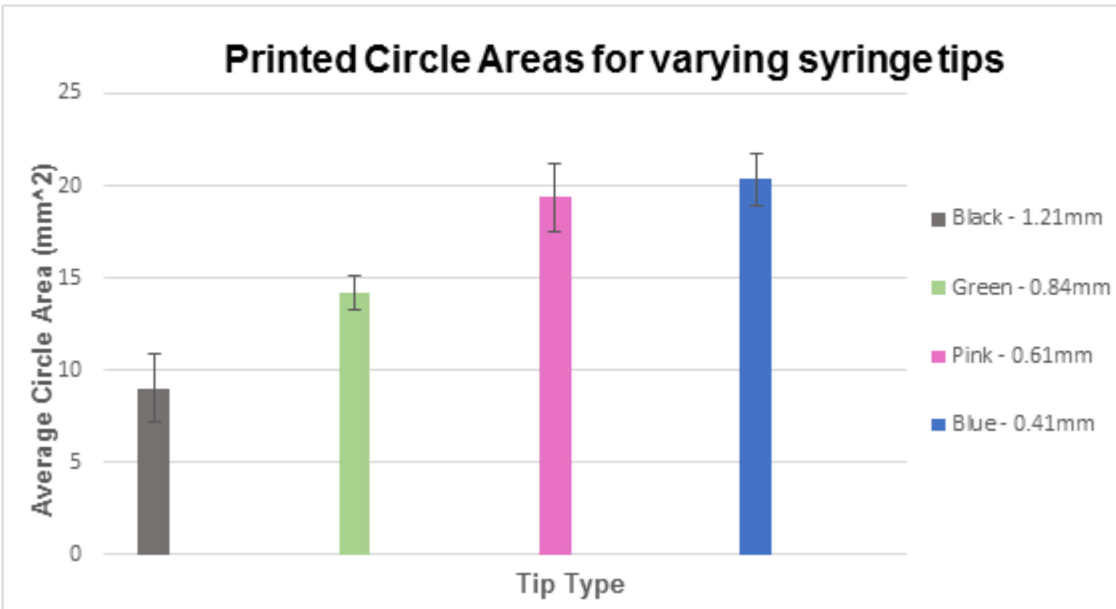
Using the four different colored/sized tips available in the lab, we printed 2 replicates for each tip under the **tB1** printing parameters on June 5 (trial names tT1-tT8).

Our findings were summarized as follows:

Figure A8: Cropped images of prints for varying tip sizes. (File tips_testing.gcode)

	Black Tip	Green Tip	Pink Tip	Blue Tip
6mm 0.5mm thickness 4 revolutions				

Figure A9: Data summarizing printed circle areas for varying tip sizes. Standard deviation calculated from replicates. [1]



The data found that the pink and blue tips showed similar outputted circular areas. In addition, they also used the smallest amount of PMMA per print. However, the blue tips needed to be replaced more frequently between prints as the smaller tip size meant the holes dried up and clogged faster. For this reason, we felt the best tip was still the pink and thus continued to use it for all future prints.

On June 11, we conducted additional 96-well prints with a pink tip and the tB1 parameters. They are identical to prints conducted in the past, showing a printing time of 12 minutes, 33 seconds and approximately 0.3mL of PMMA (Formula B) per print.

<u>Discussion:</u>

Lowered Platform

- Used the screws and springs beneath the platform to adjust the platform height
- Scraped out some excess PMMA from the syringe holder

Presence of air bubbles in syringe tips

- Reduction in air bubble issues after switching out the bottom portion of the syringe for a new piece

Leakage of PMMA Formula A (volume/volume)
- Alleviated by switching to Formula B (weight/volume)

Going Forward:

TRACER Paper Base
- Basic CAD design for the base (in progress/next week)

Printer Piece
- Awaiting email response from the BioBots company Allevi for the code (pending)

Alternative Blue Dyes
- Testing with food coloring to determine whether it can also be used as a substitute to methyl blue (next week)

References:

Dispensing Tips with Luer Lock Connection

Trim these tapered tips to the opening size you need.

Gauge	Tip ID	Lg.	Material	Color	
14	0.063"	1 1/4"	Polypropylene Plastic	Light Pink	6699A1
16	0.048"	1 1/4"	Polypropylene Plastic	Black	6699A2
18	0.035"	1 1/4"	Polypropylene Plastic	Green	6699A3
20	0.024"	1 1/4"	Polypropylene Plastic	Pink	6699A4
22	0.017"	1 1/4"	Polypropylene Plastic	Blue	6699A5
24	0.013"	1 1/4"	Polypropylene Plastic	Orange	6699A6
25	0.011"	1 1/4"	Polypropylene Plastic	Red	6699A7
27	0.008"	1 1/4"	Polypropylene Plastic	Clear	6699A8

[1] *Dispensing Tip Sizes*. (n.d.). McMaster-Carr. Retrieved from: https://www.mcmaster.com/needles

- **Dyes selection for visualizing PMMA**

We selected four types of dyes for experiment: methyl blue. Food dye, basic nail polish, no dye.

Table A4. Dyes selection and testing protocol

Dye Type	Amount	Print Pattern	Date Printed
Methyl Blue	0.001g	3mm radius, 4 revolutions, 0.5mm revlotion spacing, 3000mm/ms	2019-06-25
Food Dye	1 drop		2019-06-25
Basic Nail Polish	1 drop		2019-06-25
Clear Coat	1 drop		2019-06-25

Figure A10: Cropped images of prints for varying dyes

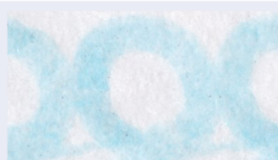
	A	B	C
Blue Food Dye			
Clear Dye(no dye)			
Blue Nail Polisher			
Methyl Blue			

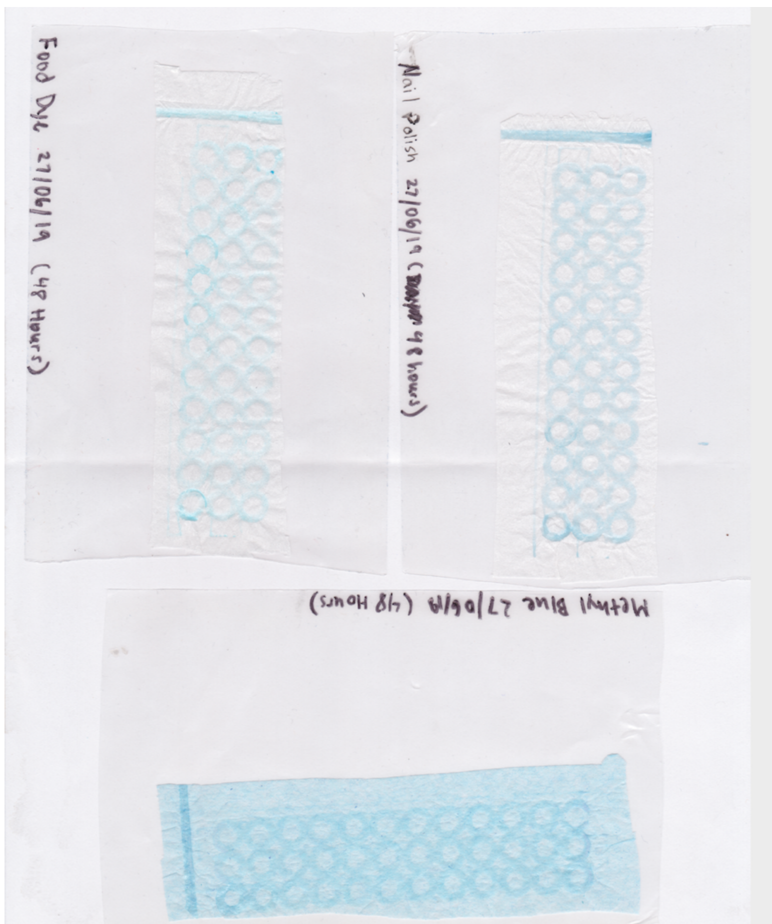
Table A5 Image processing result of different dyes

Type of Dye	Trial #	Average Area		Circularity
Blue Food Dye	A	0.027167	6	0.876
	B	0.025194	31	0.88
	C	0.026914	35	0.979
Clear Dye(no Dye)	A	N/A (can't recognize)	0	N/A
Blue Nail Polisher	A	0.027143	21	0.86
	B	0.028706	17	0.862
	C	N/A (can't recognize)	0	N/A
Mythl Blue	A	0.025618	34	0.878
	B	0.027486	35	0.889
	C	0.026611	36	0.887

Drained with PBS

Pigments were organized in a chart and the top (3) selections were chosen (Table A6)
96-well sheets dyed with 1- Methyl Blue, 2- Blue Food dye and 3- Blue nail polish were submerged in 10mL PBS and incubated in a hot water bath for 48 hours (Figure A11); Evident from visual observation Methyl Blue bled through the TRACER paper the most

Figure A11: Scanned images of 36 96-well circles (printed in final parameters in Formula) dyed with Methyl Blue, Food dye, and blue Nail Polish after 48hrs in hot water bath with PBS.



Pigment Selection

Table A6. Pigment information

Written Name	CI Number	CAS Number	McMaster-Carr	VWR	Smallest Mass Available	Price	Form	Cytotoxic?
ALUMINUM LAKE	(CI 19140)	12225-21-7	None	https://ca.vwr.com/store/product/en/9564564/food-yellow-no-4-aluminum-lake	25g	$ 172.64	Powder	Uknown(VWR)
ALUMINUM POWDER	(CI 77000)	7429-90-5	None	https://ca.vwr.com/store/product?casNum=7429-90-5	25g	$42.63	Powder	Uknown(VWR)
MANGANESE VIOLET	(CI 77742)	10101-66-3	None	None	-	-	-	-
D&C RED NO. 6 BARIUM LAKE	(CI 15850)	5858-81-1	None	None	-	-	-	-
IRON OXIDES	(CI 77491, CI 77492, CI 77499)	1309-37-1 / 1345-27-3, 51274-00-1, 12227-89-3 / 1317-61-9 / 1345-27-3	51274-00-1: https://www.mcmaster.com/1347n33	1309-37-1: https://ca.vwr.com/store/product/en/8878180/iron-iii-oxide-red	MMC: 1lb ; VWR: 100g	MMC: $25.00; VWR: $22.50	MMC: Powder; VWR: Powder	Uknown(MMC & VWR)
			1317-61-9: https://www.mcmaster.com/1347n35	**1317-61-9:** https://ca.vwr.com/store/product/en/7485567/iron-ii-iii-oxide-97-metals-basis	MMC: 1lb; VWR: 1kg	MMC: $25.00; VWR: $74.17	MMC: Powder; VWR: Powder	Uknown(MMC & VWR)
D&C RED NO. 7 CALCIUM LAKE	(CI 15850)	5858-81-1	None	None	-	-	-	-
FD&C BLUE NO. 1 ALUMINUM LAKE	(CI 42090)	3844-45-9	None	https://ca.vwr.com/store/product/en/9564556/acid-blue-9	25g	$154.69	Powder	Uknown(VWR)
FERRIC AMMONIUM FERROCYANIDE				**14038-43-8:** https://ca.vwr.com/store/product/en/9879926/iron-iii-hexacyanoferrate-ii	25g	$83.44	Unclear	Uknown(VWR)
	(CI 77510)	14038-43-8 / 12240-15-2 / 25869-00-5	None	https://ca.vwr.com/store/product/en/9883281/ammonium-iron-iii-hexacyanoferrate-ii-hydrate-tech	25g	$71.27	Unclear	Uknown(VWR)
D&C RED NO. 34 CALCIUM LAKE	(CI 15880)	6417-83-0	None	None	-	-	-	-
ULTRAMARINES	(CI 77007)	12769-96-9 / 1302-83-6 / 57455-37-5	None	None	-	-	-	-
D&C BLACK NO. 2				1333-86-4: https://ca.vwr.com/store/product/en/9881274/carbon-black-99-9-acetylene-100-compressed https://ca.vwr.com/store/product/en/9880098/carbon-black-99-9-acetylene-50-compressed	250g	$80.03	Unclear	Uknown(VWR)
	(CI 77266)	1333-86-4 / 7440-44-0	None	7440-44-0: https://ca.vwr.com/store/product/en/8869550/charcoal	50g	$10.00	Charcoal, Activated Carbon	Uknown(VWR)

since the cytotoxic is unknown for each pigment (McMaster-Carr and VWR were unable to provide cytotoxicity information for any of the pigments isolated from the nail polish), the final recommendation is made based on price.

Final Recommendations: - FD&C BLUE NO. 1 ALUMINUM LAKE (CAS#: 3844-45-9) from VWR; - IRON OXIDES(CAS#: 1317-61-9) from VWR; -D&C Black No.2 (CAS#: 1333-86-4) from VWR

Live/dead (cytotoxicity)

The resulted images are not clear enough due to the excess volume of gel added in the samples.

• B: 384-well characterization

Demonstration

We used the method of drawing squares (File: GCODE_3MM_3Lay_SQUARE .mlx ; GCODE_3MM_3Lay_SQUARE.gcode) for demonstrating the printing of 384 fit the actual well plate. Later, by switching to drawing lines instead of squares, it shortens the operation time.

Table B1. Different treatment group for characterization of 384-well

Treatment #	side length(mm)	# of Layers	Layer Spacing(mm)	Speed(mm/s)	Printed On:	# of Trials Printed
1	3	3	0.5		2019-07-22	4
2	3	5	0.3		2019-07-22	4
3	3.3	3	0.4		2019-07-22	4
4	3.3	5	0.24		2019-07-22	4
5	3.7	3	0.27		2019-07-22	4
6	3.7	5	0.16	3000	2019-07-22	4
7		1	0		2019-07-23	3
8	4.5	3	0		2019-07-23	3
9		5	0		2019-07-23	3
10	FULL(5)	3			2019-07-23	3
11	FULL(6)	5			2019-07-23	3

*Note: 6*6 layer (1,3,5) size(3-4.5); 40 prints from 11 treatment groups were printed on July 22nd and 24th;All prints were scanned and image-analyzed through ImageJ (July 24 and 25) (File: WELLPLATE.mlx ; GCODE_LINE_T1 – T9.gcode)

Figure B1. Scanned prints of 386-well

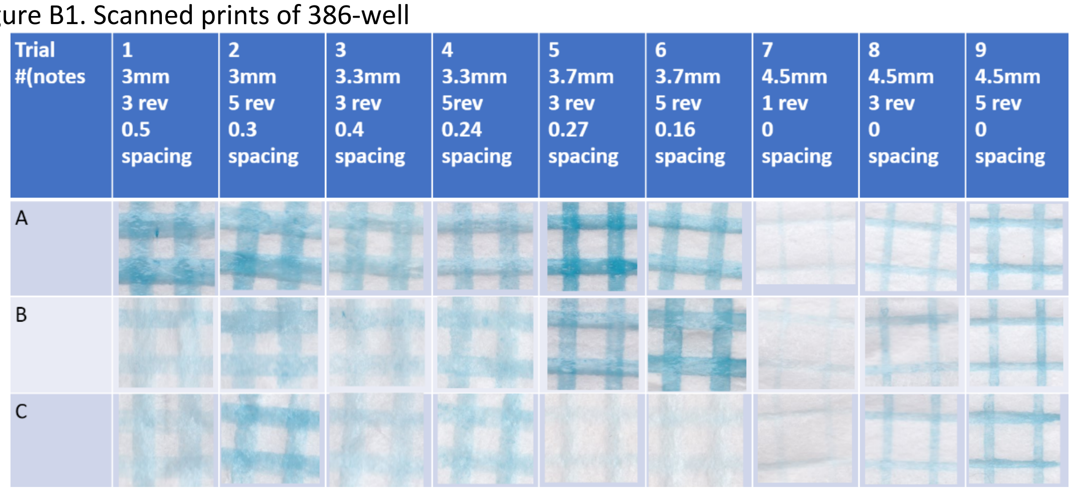
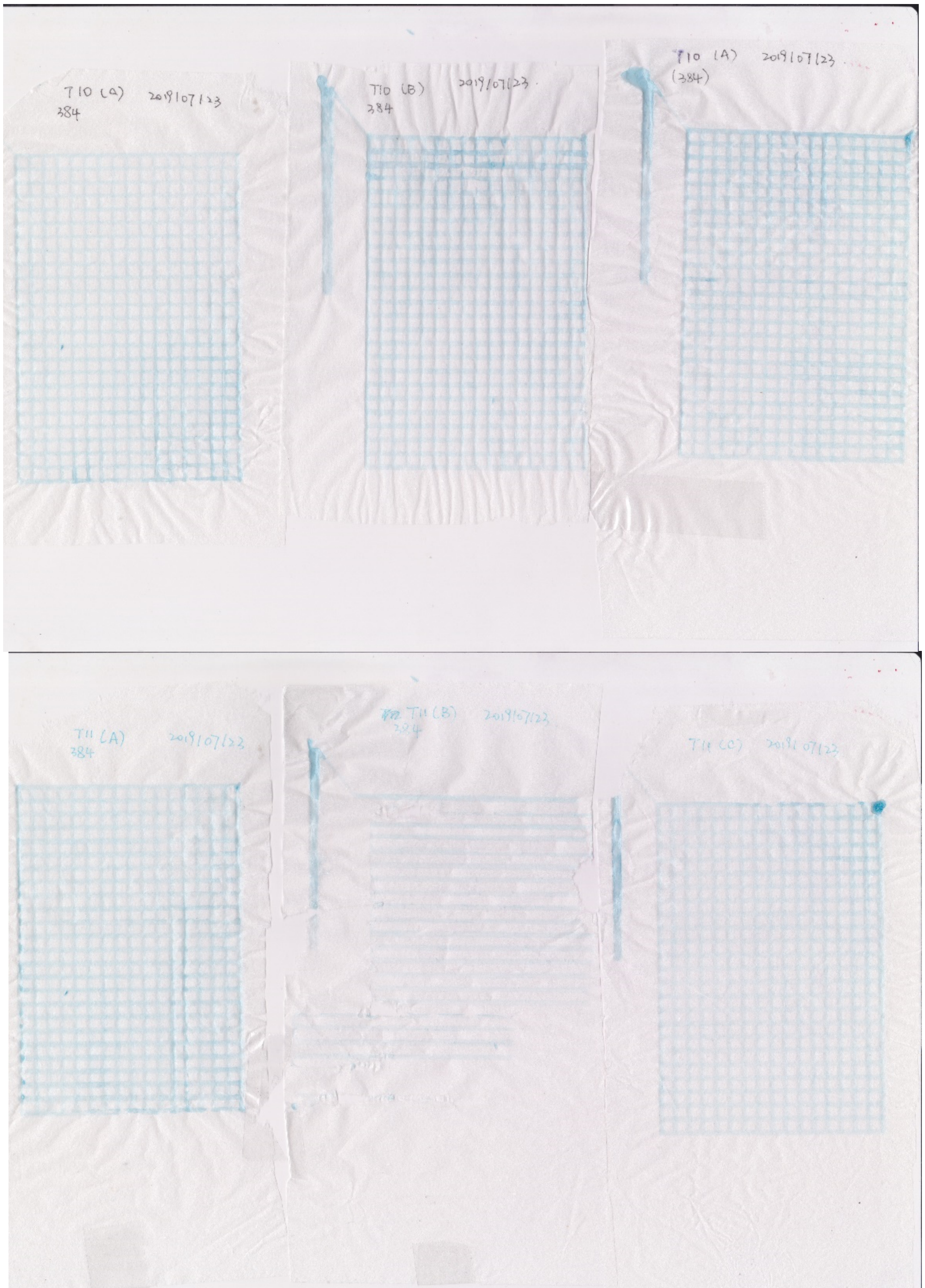


Figure B2. 384 FULL PRINT FOT T5-T6 (File GCODE_LINE_T10 – T11.gcode)



Result

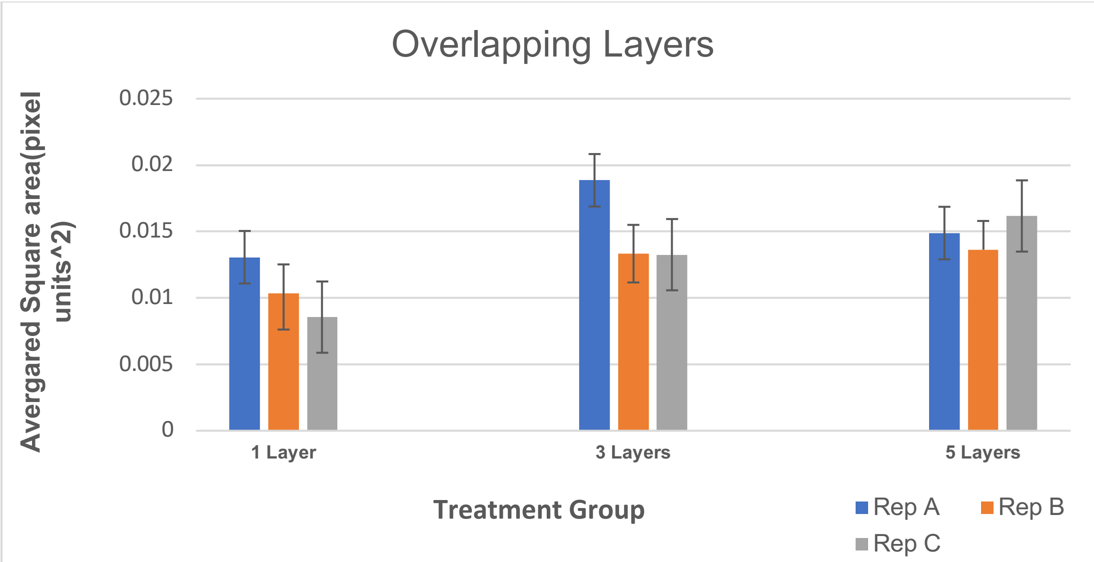
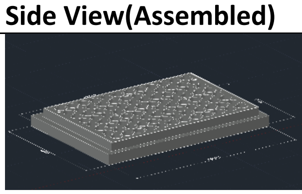
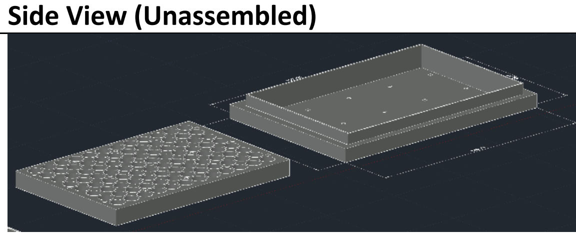
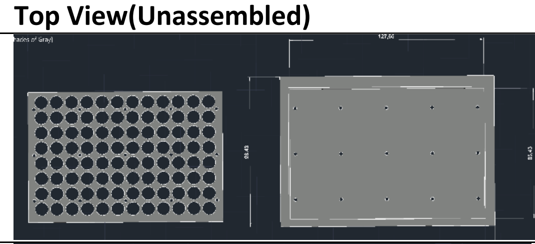
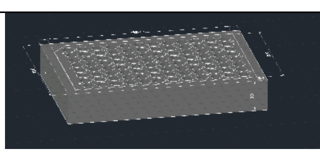
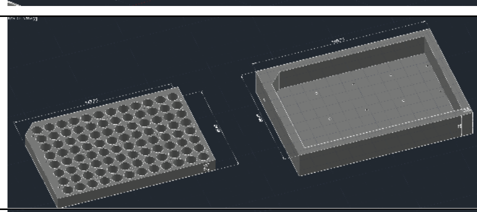
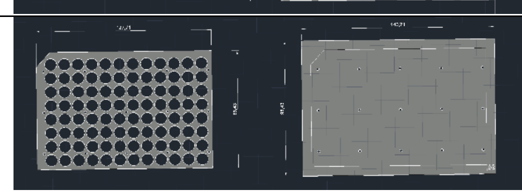
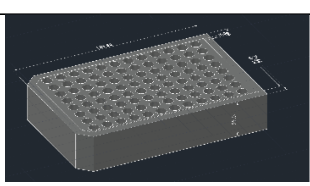
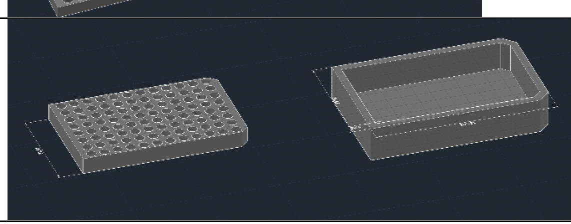
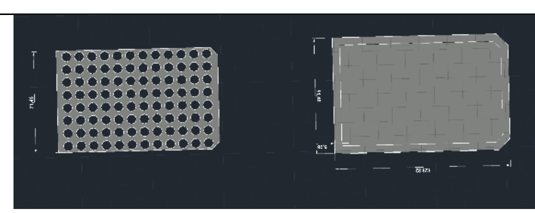
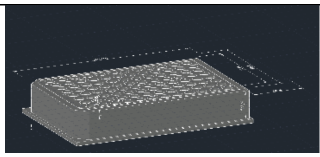
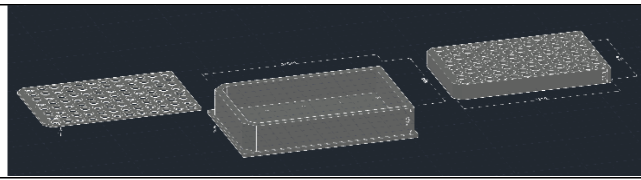
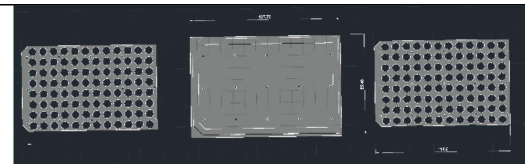


Figure B3: Square area in pixel unit^2 for 1, 3, and 5-layer overlay of Formula B PMMA pigment on a 6 by 6 grid for a 384 well plate. Each treatment group shows 3 replicates and their standard deviation.

- # C: Stacked 96-well plate
    - ### Design Stage

<u>Table C1. Designs made by AutoCAD 2019 (File: 96 well plate.dwg; 96 well plate v3.dwg; 96 well plate v4; 96 well plate v5; 96 well plate with skirt.dwg; 96-well TRACER holder 3D design ver JC.dwg)</u>

	Side View(Assembled)	Side View (Unassembled)	Top View(Unassembled)
V1			
V3			
V4			

V5			

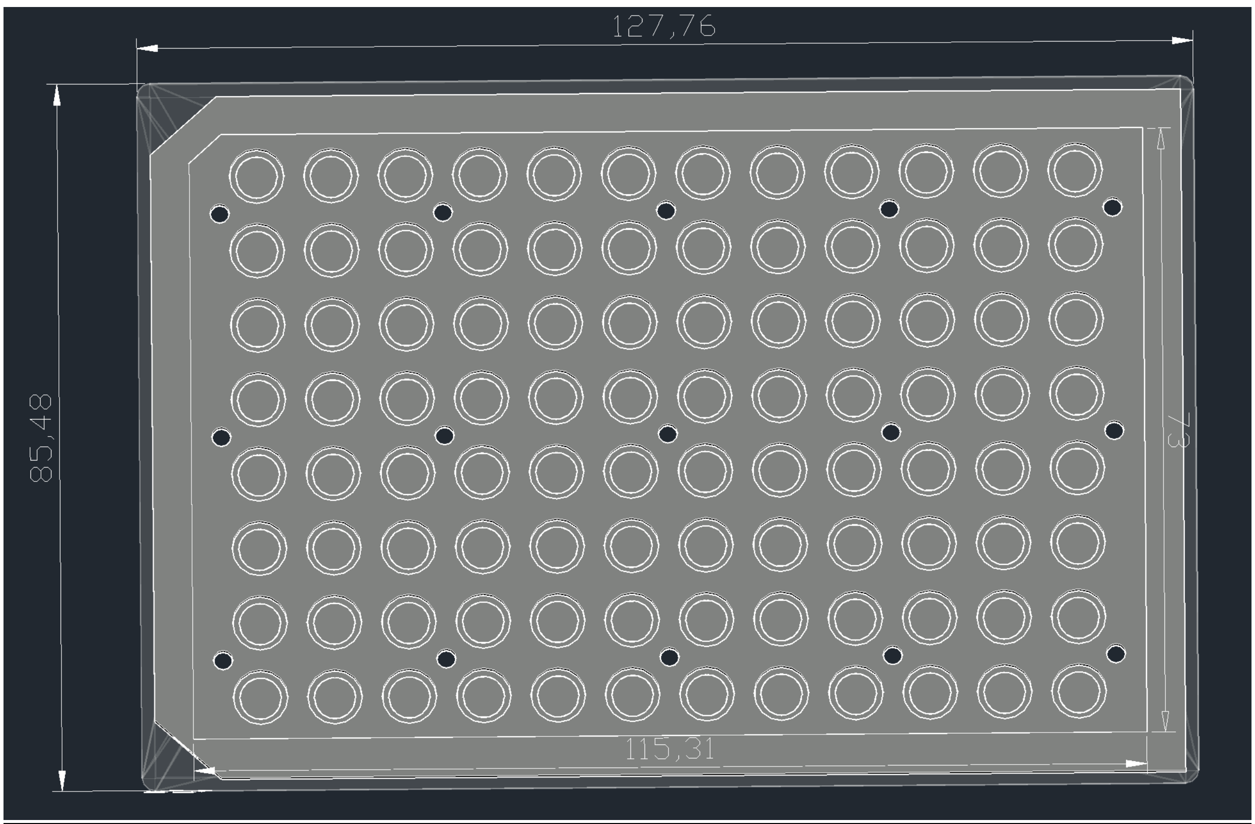
- **Comparison**

Table C2 comparison between different designs

	Dimension(length by width/mm)	Top Height(mm)	Bot Height(mm)	Skirt(mm)	Screw Diameter(mm)	Characteristics
V1	140.71*98.43(inner: 127.65*85.43)	9.5	14.6	8.1	1.9	The top part is covered with a frame and thick bottom
V3	140.71*98.43(inner: 127.71*85.43)	10.6	22	none(2mm embed)	1.9	One edge is cut to indicate direction
V4	123.82*81.48 (inner: 113.95*71.48)	11.1	23.8	none(2mm embed)	1.9	same thickness of actual well plate; two edages are cut; size adjustment in order to use same lid of the 96 well plate
V5	127.76*85.48(with skirt)123.82*81.48; inner:114.6*72.14	11.1(1.58 gasket)	19.3	1.58(2.5mm embed)	1.9	in order to use same lid of the 96 well plate
Final	127.76 * 85.48(inner:115.31*73)	11.1(1.58 gasket)	11.1	2.11(1.58 embed)	2.4	Screw Diameter adjusted to be consistent with the paper; heigt is shorten for it to observe under microscope

- **Final Design**

<u>Top view (assembled/unassembled)</u>



Bot Dimension: 127.76mm * 85.48mm

Top Dimension: 115.31mm*73mm

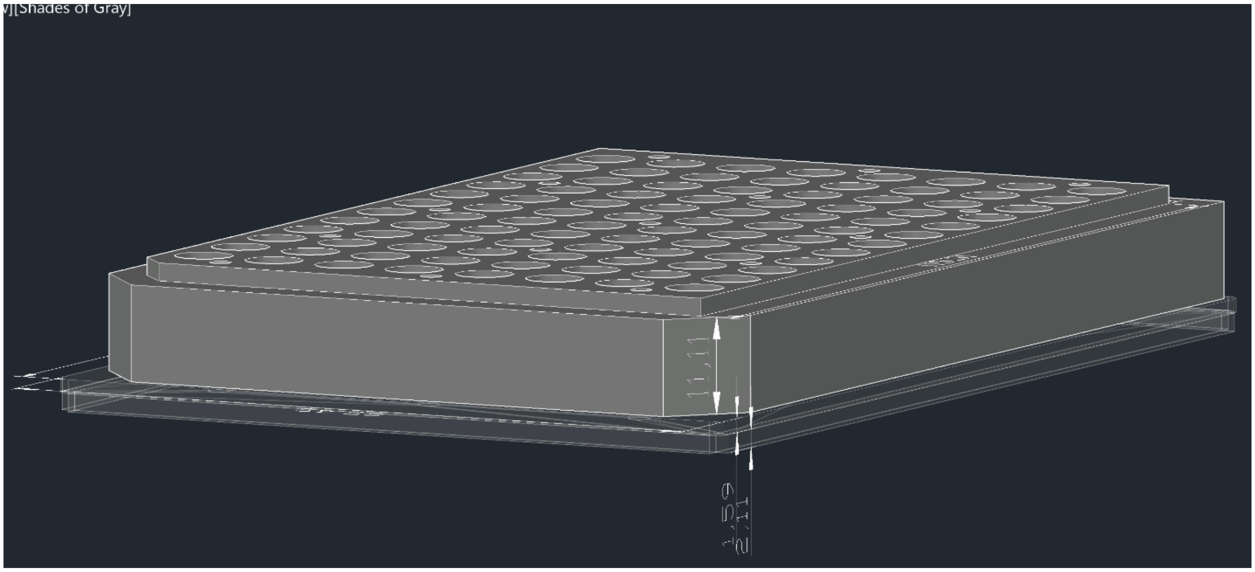
Well Diameter: 6.96mm

Well spacing: 9mm

Screw Diameter:2.4mm

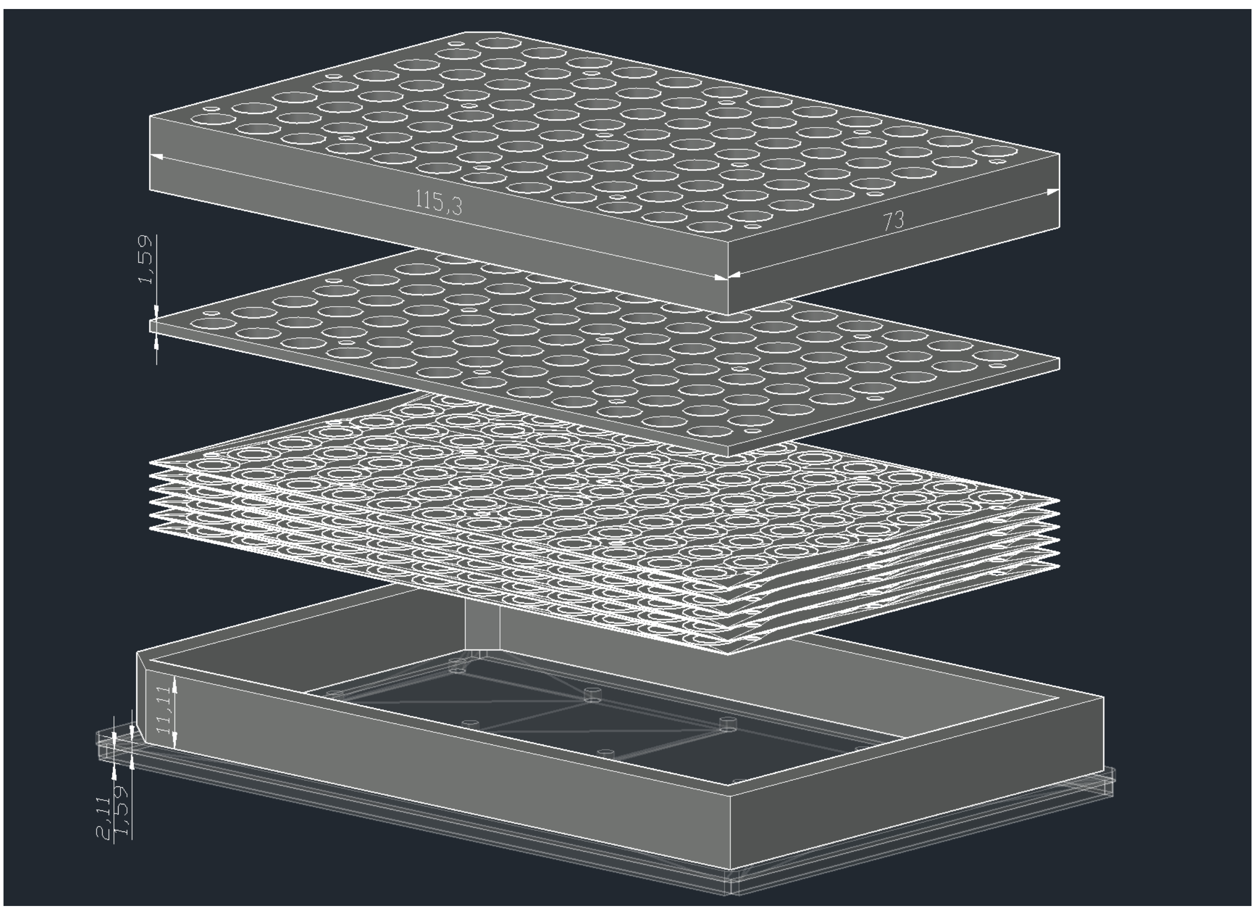
Side view

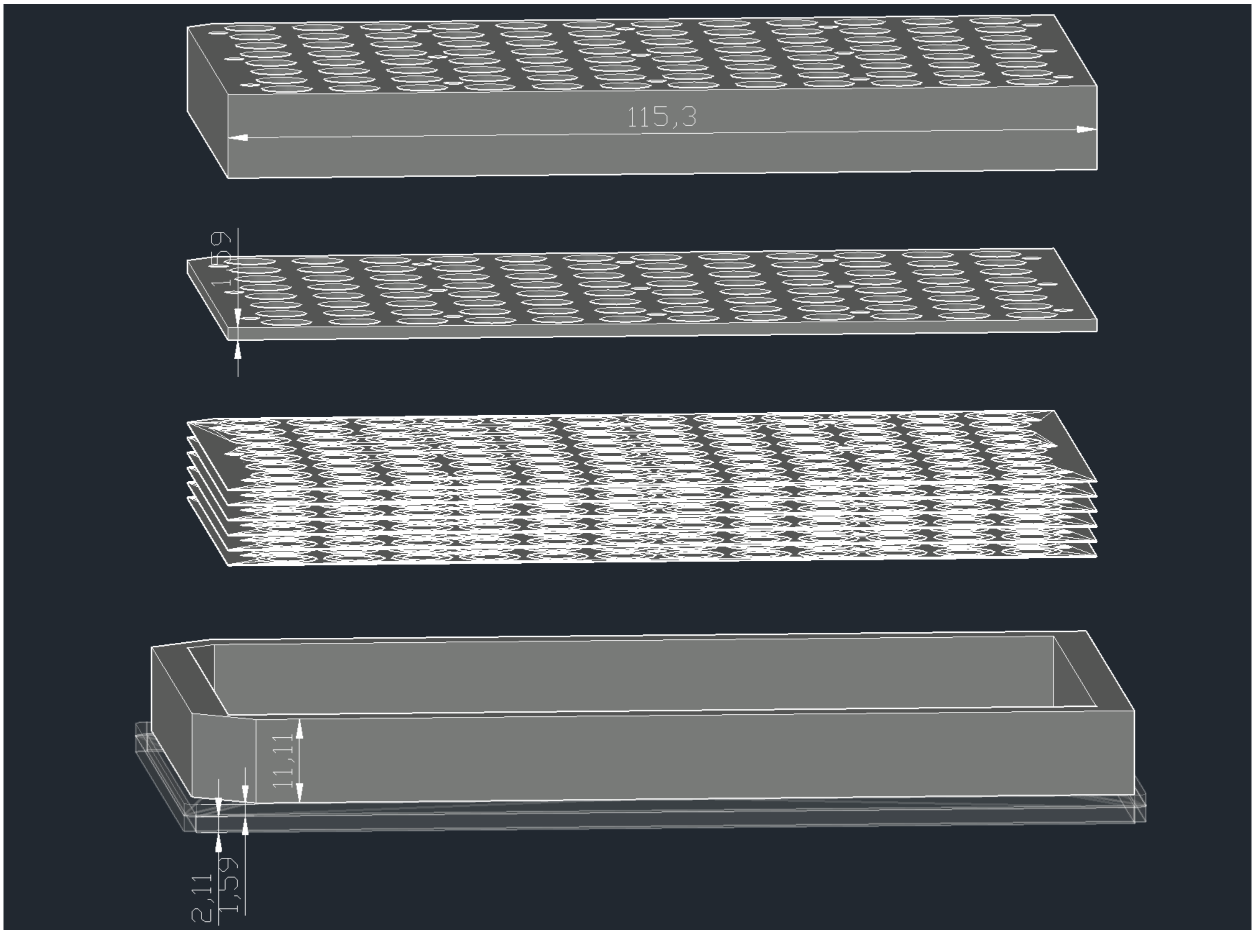
Assembled:



Bot height: 11.11mm + 1.59mm thickness + 2.11mm skirt

Unassembled:





Gasket:115.3mm*73mm*1.59mm

The designed pieces are being laser cut and assembled with 70% isopropanol

- **Leakage test**

- # D: Syringe holder
  - **Design Stage**

Put in design file name(pic)
The final design was sent to Gerstein to be 3D printed with PLA plastic

- **Testing Stage**

- # E: Seeding scaffold pipeline
  - **Gel optimization (2 µl,2.5 µl,3 µl)**

Rationale: Current gel volume used in LIVE/DEAD tests (5.0 µL) to check cytotoxicity near interfaces is too large to get a viable image from the microscope.

Treatment Groups

Treatment 1:
2.0 µL

Treatment 2:
2.5 µL

Treatment 3:
3.0 µL

Procedure:

- Seed cells with the varying gel concentrations in the PMMA wells
- Allow cells to incubate in wells for 48hrs
- Image PMMA with microscope

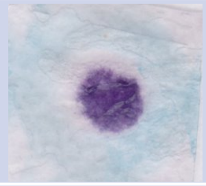
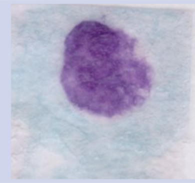
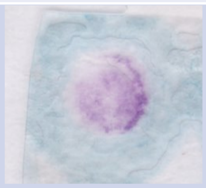
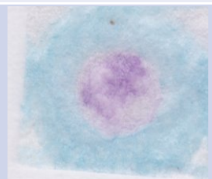
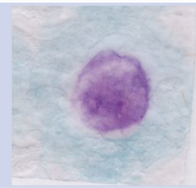
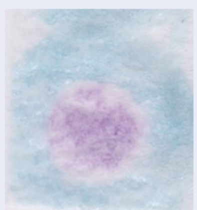
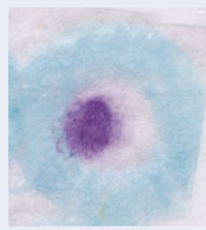
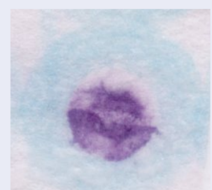
Table E1. Seeding Protocol

	Treatment Groups	# of Cells	volume of collagen(mircrolitres)	amount of MTT		Notes	Spreading	# of 4-squares
								1
	1	100000	2.00	std				1
	2	125000	2.50	std				1
	3	150000	3.00	std				1
(+)ve control	4	125000	2.5	0		no MTT		1
(+)ve control	5	125000	2.5	std(left longer)		max MTT	Spread	1
(-)ve control	6	250000	5	std			evenly	1
	7	100000	2.00	std				1
	8	125000	2.50	std				1
	9	150000	3.00	std				1
(+)ve control	10	125000	2.5	0		no MTT		1
(+)ve control	11	125000	2.5	std(left longer)		max MTT		1
(-)ve control	12	250000	5	std			no spreading	1
		1750000						
	total (including extra):	2275000	45.5					

*Treatment group 10,11,12 are not necessary and eliminated during the experiment
After 24 hours in the incubator, we add MTT for each treatment (50 µl for STD, 100 µl for max)

Result

Figure E1. Cropped images of each sample after 2 hours in the media

Parameter	A No cell Std MTT	B 2.5 µL Gel Max MTT	C 5 µL Gel Std MTT
Control			
	A 2 µL Gel Std MTT	B 2.5 µL Gel Std MTT	C 3 µL Gel Std MTT
Evenly Spread			
Unevenly Spread			

According to Figure E1, the control has indicated that no cells with standard MTT has no color, where average amount of gel with max MTT indicate dark purple. The sample with 5 µl has shown dark purple, which might be inappropriate amount. Evenly Spread with 3 µl of gel indicate a decent purple, where all with unevenly indicate darker purple. Therefore, 3 µl is optimal as a result of this experiment.

- ## F: Other random experiment
  - **Cell passaging**

We helped Nila with passaging her cells CAL33 for the month of June; and Jose's KP4 during month of August.

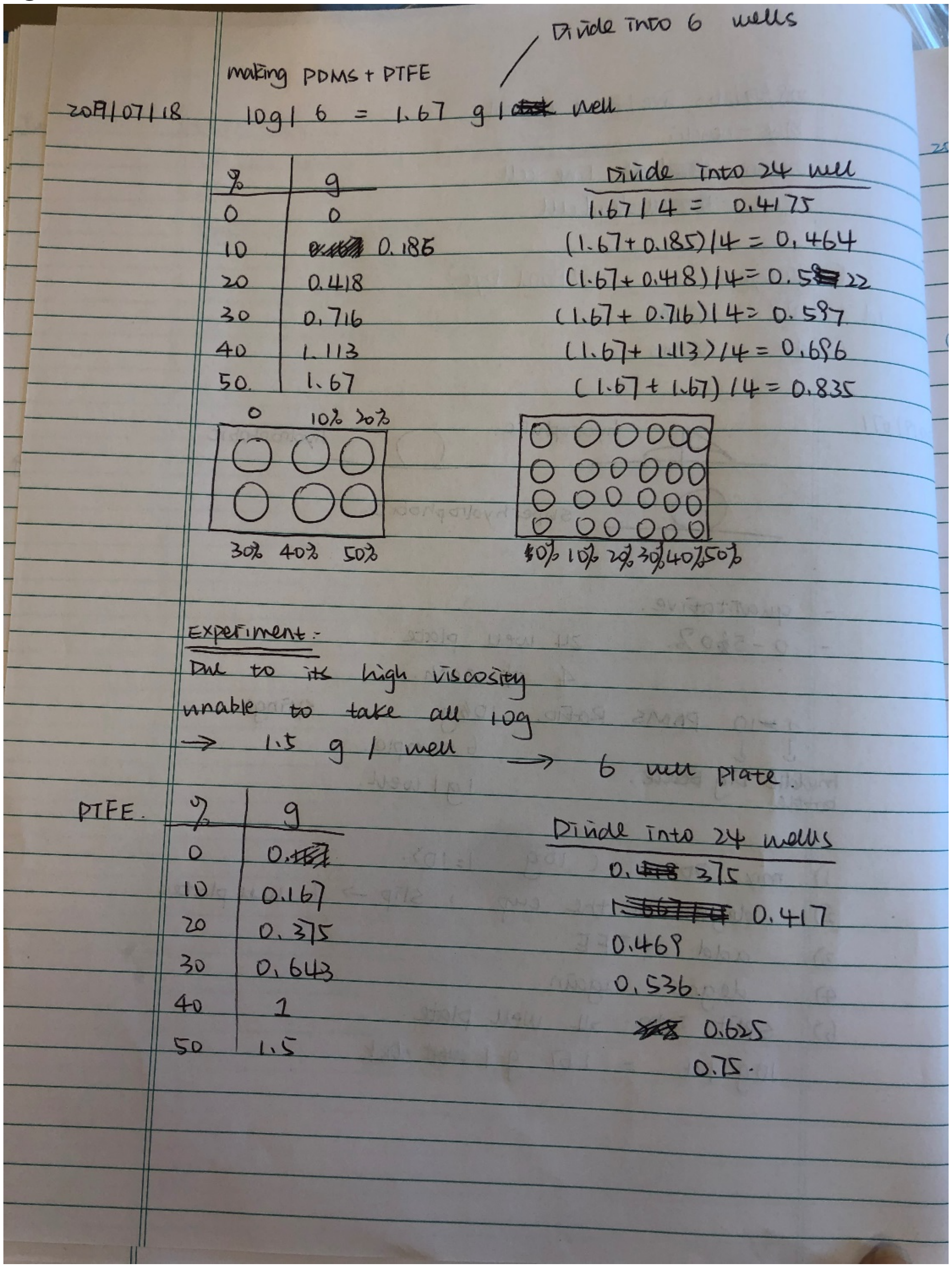
  - **Counting cell**

We also helped Natalie with hr experiment on testing protocols for digesting KP4 cells out of the collagen/material blend, which tries to maximize the number of cells that can recover for downstream analysis. The process is done by seeding TRACER Paper discs with a known amount of cells and then counting the number of cells get out at the end. We helped her preparing the different digestion solution/conditions, as well as counting the cells retrieved from each of the disc.

  - PTFE-PDMS hydrophobic coatings

We firstly mix PDMS in a ratio of 1:10, then degas the solution in the cup; After that, we divide them into 6 well plate with the same amount of PDMS and add PTFE respectively (Figure F1). Then degas again and split into 24 well plate (4 replicants for each sample)

Figure F1.Calculation for PTFE



Divide into 6 wells

making PDMS + PTFE

2019/07/18 10g / 6 = 1.67 g / well

%	g
0	0
10	0.185
20	0.418
30	0.716
40	1.113
50	1.67

Divide into 24 well

1.67 / 4 = 0.4175

(1.67+0.185)/4 = 0.464

(1.67+0.418)/4 = 0.522

(1.67+0.716)/4 = 0.597

(1.67+1.113)/4 = 0.696

(1.67+1.67)/4 = 0.835

0 10% 20%

30% 40% 50%

0% 10% 20% 30% 40% 50%

Experiment:

Due to its high viscosity unable to take all 10g

→ 1.5 g / well → 6 well plate.

PTFE.

%	g
0	0
10	0.167
20	0.375
30	0.643
40	1
50	1.5

Divide into 24 wells

0.375

0.417

0.469

0.536

0.625

0.75.

***Note during the experiment, the amount has been slightly adjusted due to difficulty in pipetting PDMS.**

Project motivation/description:

- Develop a higher-throughput stacked TRACER platform which is compatible with the 96 well-plate format
- Stacking 6 layers of TRACER scaffold which have regions blocked off with PMMA
- Preliminary characterization of various parts of the device (custom well plate, scaffolds, syringe holder, seeding device/pipeline)
- Need to perform a demo-experiment showing the utility of the system (i.e. invasion assay?)
- Expansion toward a smaller (384 well plate) design for other studies (and find possible uses for this design)

Project components:

1. **Printing 96-well PMMA disks**
   - [x] Tip size characterization and selection
   - [x] G-Code (Automatically generated with MATLAB based on printing settings that we want)
   - [x] Print pattern characterization (# of revolutions, line spacing, etc)
   - [x] Reproducibility of printed disks
   - [ ] Dyes for visualizing PMMA
     - Dye selection
     - LIVE/DEAD (cytotoxicity near interface)
2. **Seeding scaffold pipeline**
   - [ ] Gel optimization - TEST WITH 2uL, 2.5 uL, 3 uL
     - Methylene Blue Collagen disks (wicking vs manually spreading)
   - [ ] Design a seeding pipeline
   - [ ] MTT of seeded scaffolds and characterization of seeding pipeline
   - [ ] Confirm hypoxic gradient
   - [ ] Demonstrate utility of system
3. **Syringe holder**
   - [x] 3D printed CAD design
   - [ ] Test 3D printed syringe
4. **Stacked 96-well plate**
   - [x] CAD design of each PMMA piece
   - [ ] Laser-cut and assemble device
   - [ ] Leak test (single layer vs full stack)