# Laser Experiments

To obtain a biological QR code we came up with three plans depending upon the complexity and ease of use. The plans were as follows:

- Laser engraved agar plate
- Stamping
- Bio printing

Prior to having our own 3D printer, it was necessary to come up with a contingency plan to have a proof of concept ready. To achieve this, we started off by using a 2D laser CNC machine, which later was also optimised for bio printing.

There were certain challenges to overcome while working with laser CNC. For the lack of 3D printer, we decided to do engraving as well as generating stamps for the stamping process using the CNC machine. We encountered the following challenges while working on these ideas:

- To determine the right combination of speed of axial movements and the intensity of laser.
- To come up with suitable media composition as to kill necessary bacteria but keep heat diffusion to a minimum.
- Determining the appropriate material for engraving stamps.

Before we go further here are the specs for our laser CNC machine

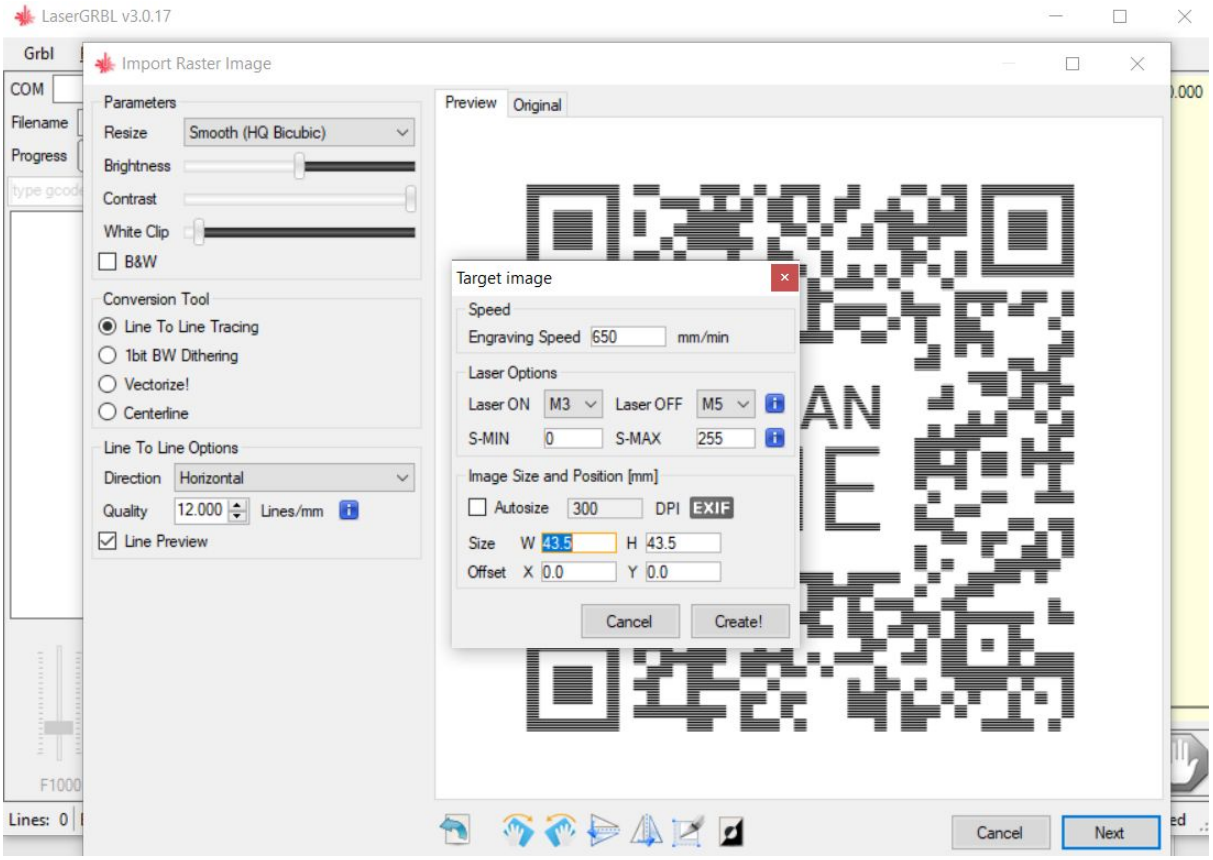
| General Specification | |
|---|---|
| Brand | EleksMaker |
| Model | EleksLaser A4 Standard |
| Frame Material | Acrylic + Aluminium |
| Max. Engraving Area | 300 x 200mm / 11.81 x 7.87 inch |
| Machine Size (L*W*H) | 460x470x150mm / 18.11x18.50x5.91inch |
| Certification | CE, FCC, FDA |
| **Technical Parameters** | |
| Control Board | EleksMana SE |
| Communication Port | MircoUSB |
| Stepper Motor | 42H34S-1304A |
| Power Input | AC100-240V |
| Working Voltage | DC 12V |
| Working Current | DC 5A |
| Laser wavelength | 400-450 nm |
| Power | 500 mW |
| Supported Formats | jpg, bmp, svg, G-code |
| Engraving Accuracy | 0.01mm |
| Support Systems | Win XP / 7 / 8 / 10 |
| Support Software | EleksCam, Candle, T2 Laser, GRBL Controller, LiteFire, etc. |
| Engrave Mode | Photo, Word, Scan, Outline, Pixel Laser Engraving |

## Engraving the agar plates:

For the testing, our wet team gave us a few agar plates with E. coli to overcome our first hurdle of determining the right combination of speed and intensity. Also, they made a special black agar in pursuit of making agar stamps.


*link and pics*

The Agar being transparent to a certain degree along with the see through disposable plates, we soon discovered that it wasn't necessary to consider the intensity of the laser as a variable parameter but rather we could make things works by controlling the "Engraving speed (speed of axial movements)" and "Quality (number of lines/traces per mm)" a feature from Laser GRBL opensource software to manipulate our results.




After performing a couple of experiments and realizing we were under equipped to have a spatial heat diffusion measurement in the agar plates, we thought of graphical extrapolation from the data we had to come up with an effective combination of speed and quality.

Following table represents the preliminary experimental data

| Exp. # | | Agar | Container | Method | Laser Iterations | Bacteria | Results | Size [mm] | Speed [mm/min] | Density Lines [lines/mm] (1-20) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | Clear | Plastic | Line To Line | 1 | E.Coli | No Effect | 55x55 | 400 | 2 |
| 2 | | Clear | Plastic | Line To Line | 1 | E.Coli | No Effect | 55x55 | 200 | 2 |
| 3 | | Clear | Plastic | Line To Line | 1 | E.Coli | Positive | 40x40 | 200 | 10 |
| 4 | | Clear | Plastic | Line To Line | 1 | E.Coli | Bad Density QR | 40x40 | 50 | 2 |
| 5 | | Clear | Thick Paper Stamp | Line To Line | 1 | E.Coli | No Effect | 32x32 | 600 | 20 |
| 6 | | Clear | Thick Paper Stamp | Line To Line | 1 | E.Coli | No Effect | 30x30 | 700 | 10 |
| 8 | | Clear | Plastic | Line To Line | 1 | E.Coli | Positive | 30x30 | 200 | 7 |
| 9 | | Clear | Plastic | Line To Line | 1 | E.Coli | Scannable | 30x30 | 250 | 12 |

Now for extrapolation we will only use speed and density(lines/mm)

| Speed [mm/min] | Density Lines [lines/mm] (1-20) |
|---|---|
| 400 | 2 |
| 200 | 2 |
| 200 | 10 |
| 50 | 2 |
| 600 | 20 |
| 700 | 10 |
| 200 | 7 |
| 250 | 12 |

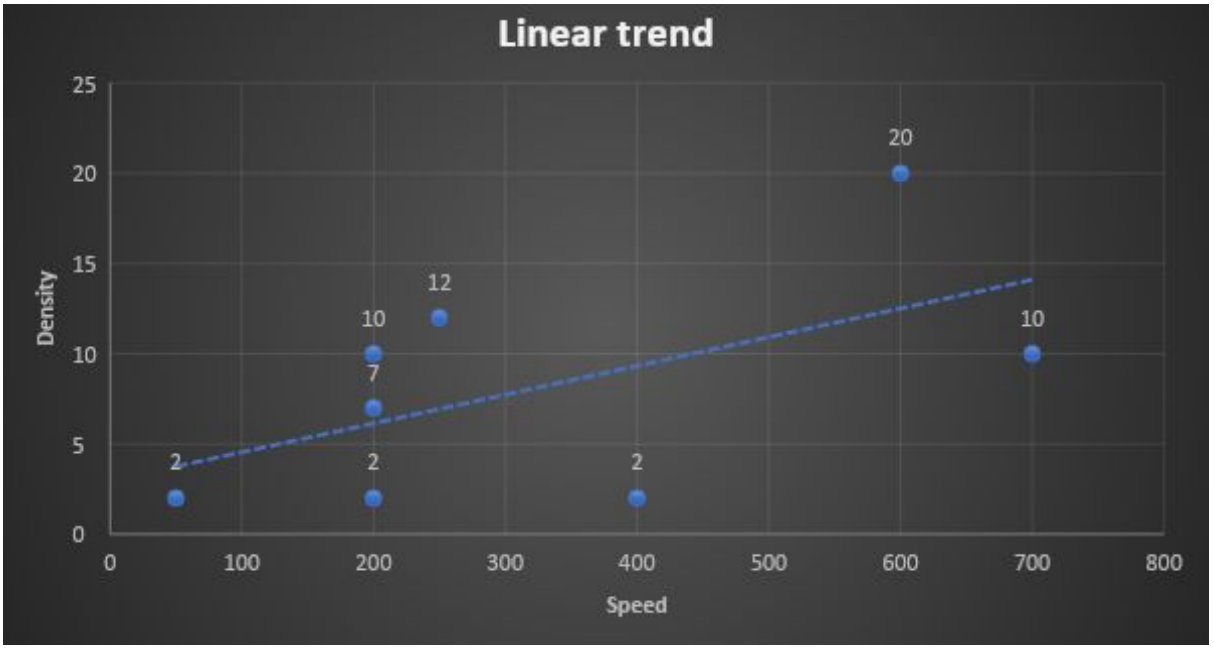
Since we did not know the exact nature of heat diffusion in agar, we did three simulations based on three trends:

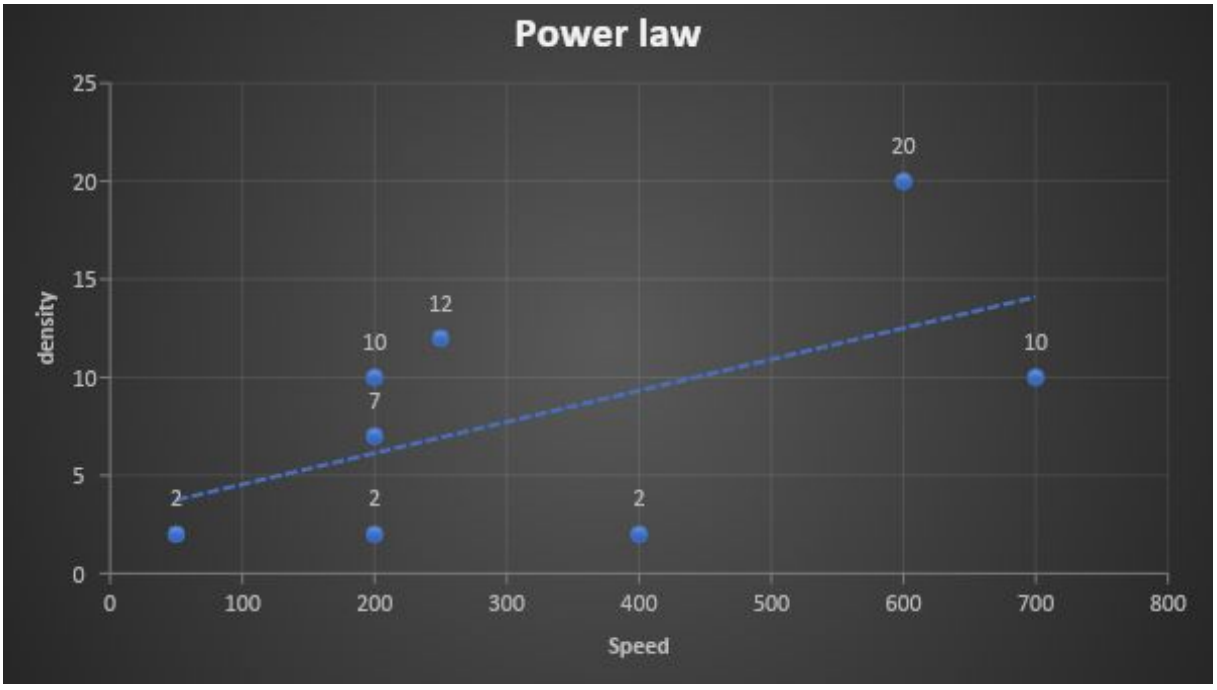
- Linear
- Power law

We skipped exponential trend since from Stephen-Boltzmann's law, we know that the net rate heat transfer between one radiating body and one absorbing body is given by

$$q = \sigma A_1 \varepsilon_1 (T_1^4 - T_2^4)$$

Thus, we can observe the trends graphically as follows:




From these graphs we were able to extrapolate a favourable thumb rule for clear agar plates which can be represented as

$$\frac{Speed}{Quality} \sim 20.5$$

Exact values of speed and quality cannot be determined because of other variables like thickness of agar, constitution of media and size of the plate.

Conclusions:

The engraving sure did give positive results as follows but we also came understand a couple of drawbacks.

*insert pics here*

Drawbacks of this method are:

- Use of multiple strains on a same plate doesn't work.
- The QR code must be generated in a plate and then sealed off for containment.

## Material tests for stamping:

To follow up with our second plan of stamping first we decided to engrave stamps using the laser engraver. To accomplish this we started to tests different materials with our CNC machine to find the right material for the stamps.

| Materials | Speed (mm/min) | Density (lines/mm) | Type | No of runs | Results | Comments |
|---|---|---|---|---|---|---|
| black divider film | 600 | 20 | L2L | 1 | failed bad | melting the material, nonlinear, bad resolution |
| rubber | 200 | 12 | L2L | 1 | miserable failure | laser has insufficient power to even etch the rubber surface |
| canvas square | 50 | NA | vector | 1 | no depth | paper on top, no good for stamping, no depth in engraving |
| cork coaster | 2000 | 20 | L2L | 1 | gift shop etching | burns quickly, very low resolution (crooked edges) |
| ***WOOD!!!*** | ***600*** | ***20*** | ***L2L*** | 1 | ***MAGNIFIQUE*** | good depth, high resolution, sharp edges |

Hence, we decided to go with wood as a preliminary stamping material.

Testing different speed and density on Wood for depth and resolution of the stamps

| Size (QR) (mm^2) | Speed (mm/min) | Density (lines/mm) | no. of laser runs | Results |
|---|---|---|---|---|
| 20x20 | 600 | 20 | 1 | Magnifique, less depth |
| 25x25 | 500 | 20 | 1 | good, less depth requires intense cleaning to be scanned |
| 25x25 | 600 | 20 | 1 | awesome, good depth clean edges perfect for stamping |

But soon we realized that the soft wood we were using faced a few problems:

- The wood tends to bend after a couple of dips into bacterial solution
- The generated QR has low resolution
- Wood absorbing the solution can be critical to safety protocols

Next we moved to making stamps with black agar plates. Since its infusion with charcoal we though the heat diffusion would enhance the melting of the agar and provide us with good resolution and quick stamp build.

| **Size** | **Agar** | **Plate** | **Type** | **Runs** | **Bacteria** | **Results** | **Speed** | **Density** |
|---|---|---|---|---|---|---|---|---|
| 30x30 | Black | Plastic | Line to Line | 1 | E.Coli* | Kills but melts agar | 250 | 12 |
| 30x30 | Black | Plastic | Line to Line | 1 | E.Coli* | Melted agar | 800 | 12 |
| 55x55 | Black | Plastic | Line to Line | 1 | E.Coli* | No Effect | 1000 | 12 |

As we can see due to lower tuning capacity of our laser, we were unable to forge good quality black agar stamps.

## Stamping

Since we settled on using wood as a favourable material for stamping, we started testing different speed and density on Wood chips for depth and resolution of the stamps

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As we can see due to lower tuning capacity of our laser, we were unable to forge good quality black agar stamps. Meanwhile we received our 3D printer and hence we shifted from wood stamps to 3D printed pixelated stamps.

These 3D printed stamps were designed on open source software and used by the wet team for direct stamping and velvet replication techniques.