Advancing behavioral arenas for small model organisms by 3D printing nematode growth medium (NGM)

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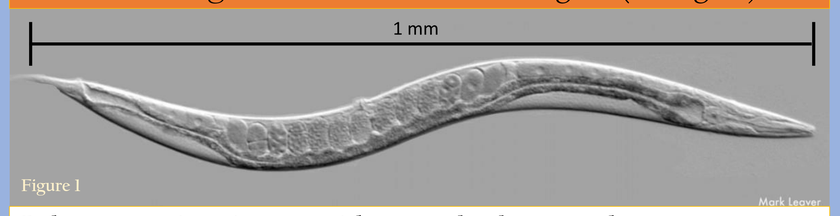
Model organism: *Caenorhabditis elegans (C. elegans)*

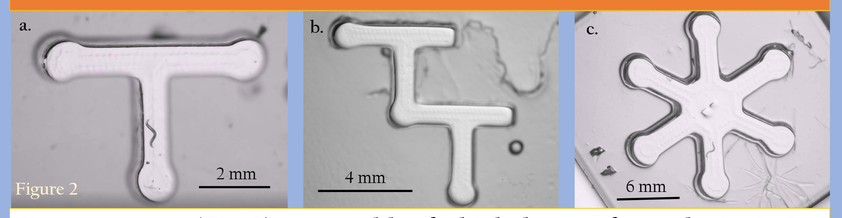


Only creature in existence with a completely mapped nervous system of ~300 neurons (Fig. 1)

95% homology with human genes makes conclusions relevant to higher organisms, sequenced genome, short lifespan, several genetics tools

Shown to be able to learn [2] (associative, non-associative learning)

Current Behavioral Arena's Standard

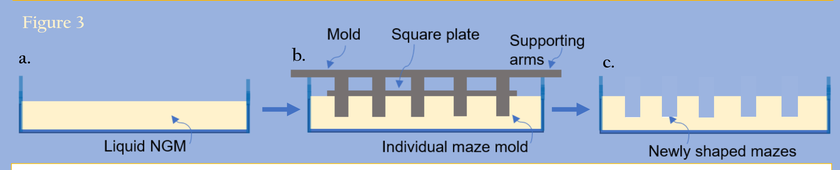


Current arenas (Fig. 2) are capable of a high degree of complexity in 2 of the 3 spatial dimensions (X and Y, but not Z)

Able to obtain exciting results about decision making & learning [1]

C. elegans are cultured on NGM plates, so **all arenas need to be made of NGM as well**, to minimize stress due to environmental change

Current Manufacturing Method-Limitations

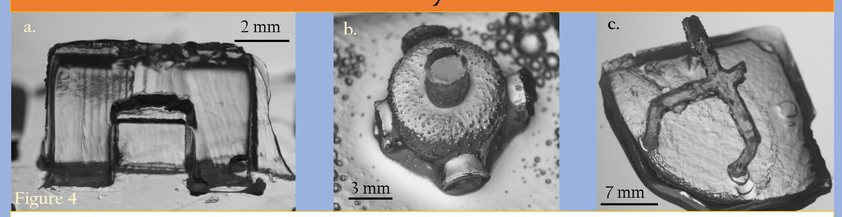


NMG: agar-based hydrogel, enriched with nutrients, liquid above **45°C**. All processing (Fig. 3) is done at room temperature [1]

Constrain: Removing the mold after NGM solidifies requires a linear pullout, which limits the degree of complexity the molds are capable of

We seek to achieve **high complexity in the 3rd spatial dimension (Z)** to explore more advanced behavioral questions

Preliminary Results



Casted arenas with **resin** molds (Fig. 4a) and **PVA** molds (Fig. 4b, 4c)

Arenas were **nonreproducible** and had severe **surface roughness** which inhibited *C. elegans* locomotion and microscopy optics

These trials proved NGM's capability to **support** itself over relatively large distances (20 mm in Fig 4c)

Design Novelty

Figure 5: Thermoplastic FDM

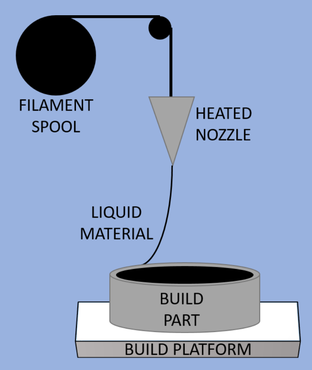
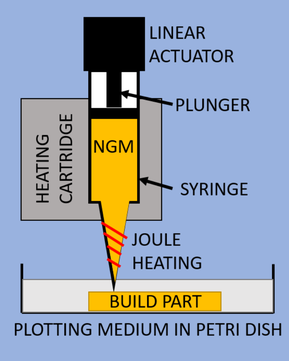


Figure 6: Thermoreversible Hydrogel FDM



Fused Deposition Modeling (FDM)

FDM: type of 3D printing consisting of **liquid deposited** on solid, **fusing together** one layer at a time in a Cartesian system (XYZ)

Gcode language: used to command FDM printers, written by 3D printing **slicers** like Ultimaker Cura. **Gcode** tells the printer where and how fast to move, and how much material to extrude

Plotting medium: For common thermoplastic FDM, air is sufficient to prevent liquid spreading & maintain shape

Our goal:
Print NGM using FDM, commanded by Gcode. A density matched plotting medium (not air) is required to prevent liquid spreading.

Methods

Solidification Kinetics-Rheology

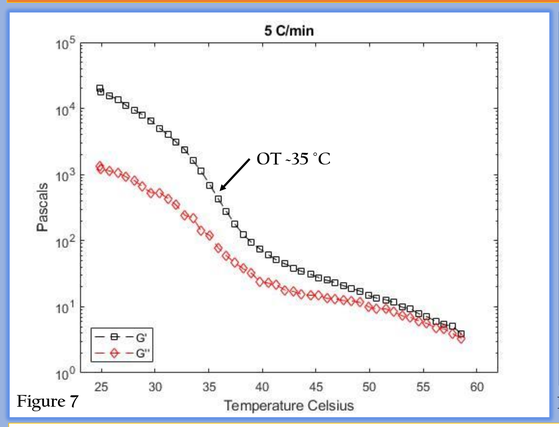


Figure 7

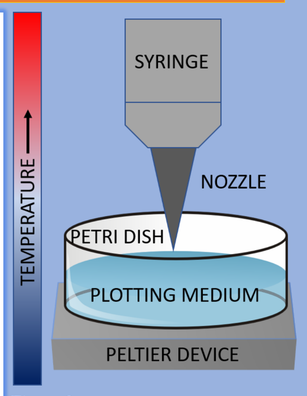


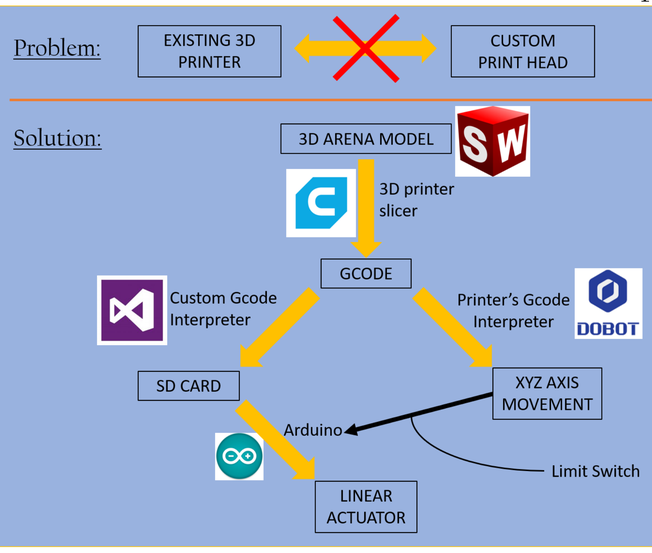
Figure 8

Fig. 7: **Rheology** tests run to determine optimal temperature (OT) to accelerate NGM solidification. OT corresponds to the steepest decline

Fig. 8: Schematic of the printing apparatus. In the syringe, NGM is kept at **~60 °C** by an Al block, heating cartridge and temperature controller and in the nozzle by **Joule** heating[3].
Plotting medium (glycerin [3]) is cooled by a **Peltier** device.

Print Sync via Mechanical Limit Switch

Lack of electrical connection makes *in-situ* communication impossible



Print head hits limit switch signaling Arduino to begin printing.

Extrusion via Linear Actuator to Syringe Plunger

The actuated arm is connected to a 12mm ID syringe plunger and by extending the arm, NGM can be extruded

Stroke (distance / step) and **Delay** (time / step): control extruded volume / time

Current model: 15.2 s layer time and 42 mm³ layer volume

Fig. 9: Number of Steps and Delay required for each stroke; Layer Volume constant, varying Target Time

Fig. 10: Number of Steps and Delay required for each stroke; Layer Time constant, varying Target Volume

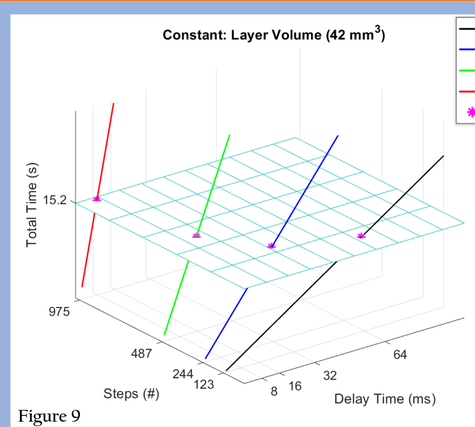


Figure 9

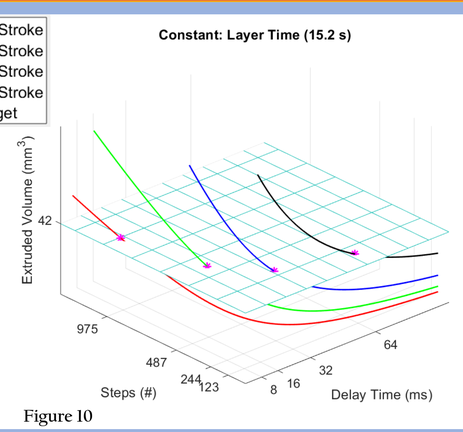


Figure 10

Results

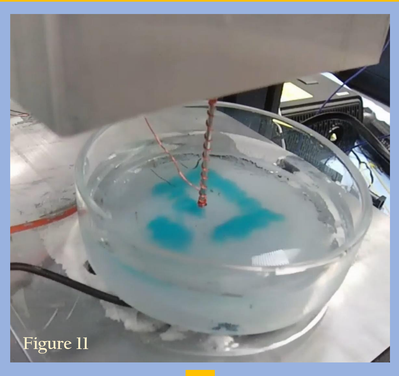
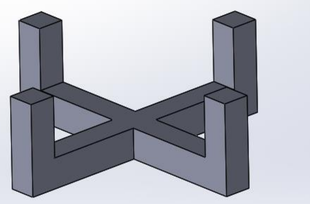


Figure 12



Current Achievements

- ✓ Nozzle with plastic Leur-Lock, 0.5 mm ID
- ✓ Joule heating resistance controlled by adjusting length of copper wire (Fig. 11)
- ✓ Plastic syringe prone to minimal size fluctuations from heat exposure
- ✓ Only works with manual gcode and actuator commands, can not slice any arena design

Future Improvements

- ➢ Lower ID nozzle to improve printed arena resolution
- ➢ Implement high precision rheostat in series for more accurate Joule heating
- ➢ Transition to a glass syringe and plunger to bypass the plasticity of current set up
- ➢ Build a machine that can take a CAD arena model (Fig. 12), slice it, and print it

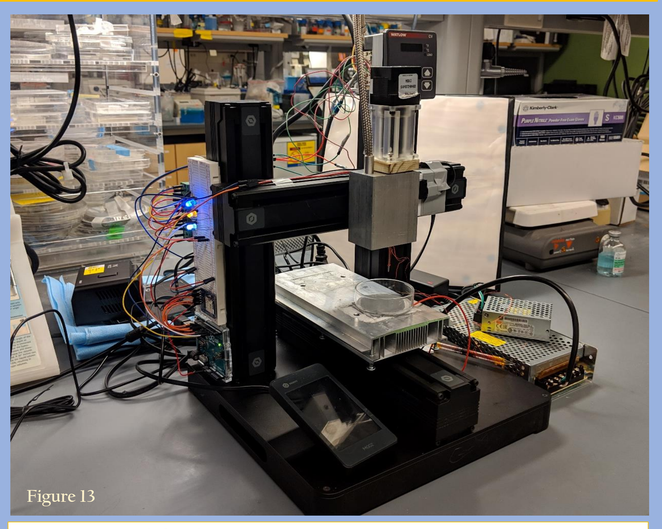


Fig. 13: Modified printer is a DOBOT MOOZ-2

¬simple **modification** ¬**gcode interpretation**
¬high axis **precision** ¬z-axis **stability**

6 items plugged in:
¬printer's axis system ¬Arduino
¬Peltier device ¬actuator
¬Joule heating ¬heating cartridge

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