Laser Cell Micropatterning: System Design and Construction

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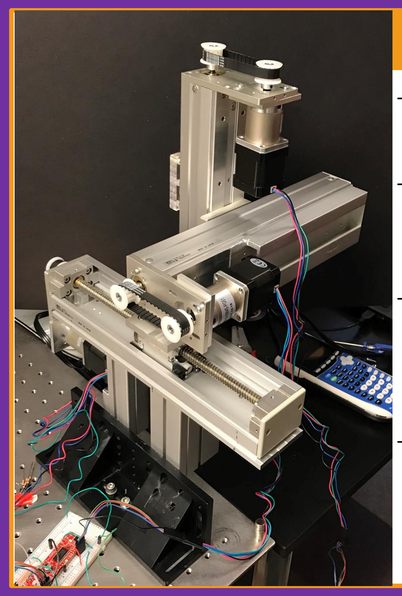
Abstract

Laser patterning cells allows for a high spatial and temporal resolution. With the practice of laser guidance, cells (e.g., epithelial) can be patterned accurately to observe their behavior under a defined microenvironment. Eventually, laser patterned cells can be added to 3D bio-printed materials. In order to achieve this, a micron accurate stage that moves in XYZ directions, change speeds and stores and returns to specific positions is needed. This research is concentrated on development and design of the Joystick controlled stage.

Background

- Ashkin(1970) showed that the radiation pressures from the focused laser beam could affect the dynamics of a micrometer sized, transparent, neutral particle. This led to the naming of the two optical forces, gradient and scattering force. As well as the use of a Gaussian beam.
- The first biological experiments were held by Ashkin and Dziedzic (1987) using a green 120mW argon laser. Viruses exceeded physiological constraints leading to the use of Infrared Light (800-850 nm).
- Laser guidance was introduced by Renn and Pastel (1988) when they successfully suspended NaCl droplets. Later Odde and Renn (2000) were able to use this system on embryonic chick spinal cord neurons giving us the first time this was used on living cells.
- Laser Guidance has a less focused objective lens, larger working area and larger working speed than optical tweezers. It yields a high spatial and temporal accuracy when compared to other patterning techniques making it ideal when working in defined microenvironments

Stage Design

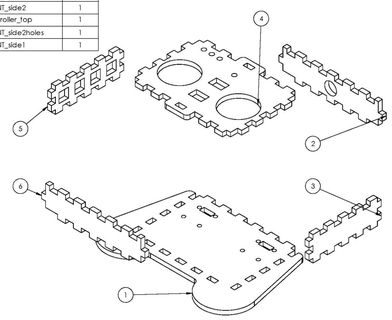


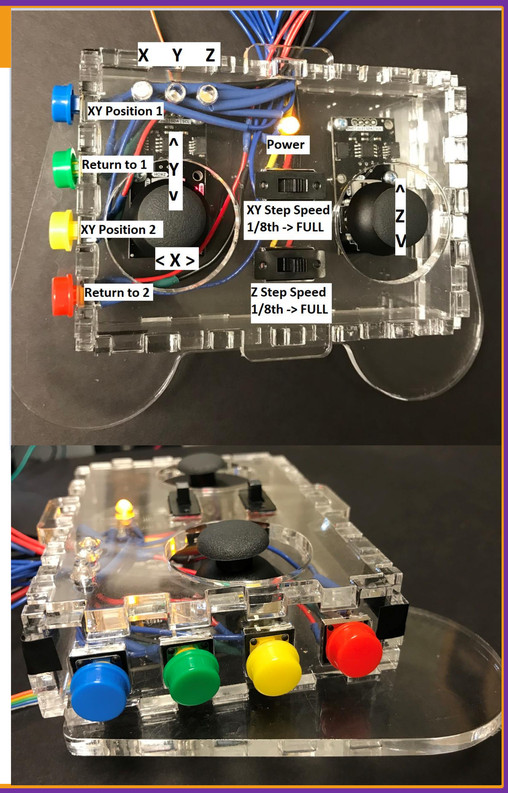
- 3 axis Translational stage with micrometer resolution
- Replaced existing stepper motors with Nema 17 51:1 gear reducer ratio
- Clemson university machine shop created custom aluminum pieces to mount the new motors to the existing stage
- McMaster-Carr supplied XL series pulleys (1” OD, 12 Teeth) and XL series timing belts, (8” circumference, 40 teeth, width 3/8”)

Joystick Controller

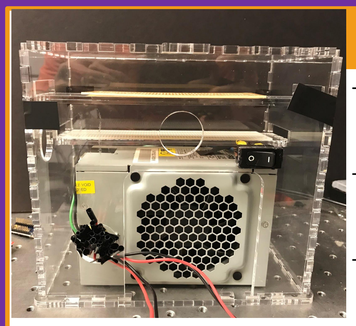
- Two Joysticks (Joystiic v1.1 - Qwiic Joystick Breakout) control XYZ directions, with LED visual cues to show which direction is active
- Two switches control speed which can be changed between two step sizes (full, half , quarter or eighth)
- Four buttons either store or return to specific positions (XY only) (Store positions: (Blue/Yellow) return:(Green/Red))

ITEM NO.	PART NUMBER	QTY.
1	controller_bottom	1
2	CONT_side1Hole	1
3	CONT_side2	1
4	controller_top	1
5	CONT_side2holes	1
6	CONT_side1	1



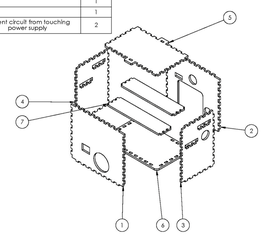


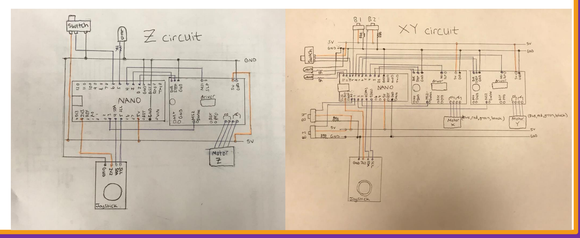
Power Box and Hardware



- Both the controller and power box are laser cut using 5mm cast acrylic
- Box contains a PC power supply (5V) soldered to a switch and all the hardware components of the system
- Hardware consists of two Arduino Nano circuits with three Sparkfun Easy driver stepper motor drivers. The Arduinos control all the electrical components including the joysticks, drivers, LEDs, and switches

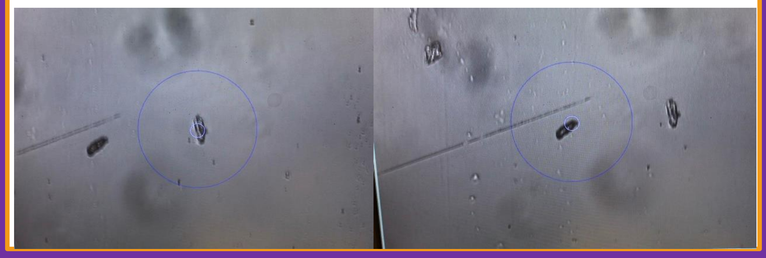
ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	Back_box	Power cable hole and vent hole	1
2	front_box	switch,vent,wire,controller holes	1
3	side1_box	board and motor wire holes	1
4	side2_box	board holes	1
5	box_top		1
6	bottom_box		1
7	board_holders	Prevent circuit from touching power supply	2





Results

- Using the previously set up laser guidance system, we imaged the use of the store and return buttons as well as tested the speed changes while using the joystick controlled stage
- The controller could accurately navigate the microscope slide at different speeds and the store and return buttons were able to maintain the same positions when toggled back and forth



Future Work

- Use Laser guidance to pattern cells (e.g., epithelial) for observing cell to cell interactions in a micro environment or to add cells to 3D bio-printed materials

Room to Improve:

- Integrate the power box and controller to clean up wires, increase overall motor movement speed, combine the two Arduinos into one

Conclusion

- The current stage model moves at an accurate, micron level
- Stored positions can easily be toggled in order to return to vital spots during experiments
- With the ability to change the speed, spatial and temporal accuracies are achieved

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