Laser Cell Micropatterning: System Design and Construction

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Introduction

In order to create a cell patterning system that has a high temporal and spatial resolution, the technique of laser guidance is employed. Cell to cell communication depends mostly on cell to cell contact and diffusible signals; that is why spatial configuration is so important. So, cell patterning methods were designed to create in vitro cell cultures that mimic in vivo patterns of cell organization.

By using laser guidance systems, we can effectively pattern cells at very specific locations in space at very specific times. Controlling these factors gives us an accurate pattern that would more closely mimic cell to cell interactions and their ability to form multi-cell systems in vivo. Placing cells at specific points in space (spatially) assures they function properly under in vivo like cell engagements and placing them at certain times (temporally) allows for the correct development of interactions

If we use the method of laser guidance we can pattern a group of epithelial cells in a row onto and existing cell culture and observe their behavior. The optical trapping forces allow us to pick up each individual epithelial cell and place them with a fast speed giving us accurate spatial and temporal results without altering the existing cell culture. With the patterning of epithelial cells, we expect to see them form a blood vessel by curving and creating the tube-like structure that is vital for blood flow.

Other cell patterning techniques, such as surface patterning and 3D bio-printing, do not have the necessary accuracy needed for systemic cell to cell interaction studies[6].

Surface patterning uses microfluidics to push a large number of cells across either a surface with physical wells to catch cells, or chemical bonding sites to bond cells in specific positions. This can create a patterned cell culture however it does not have a high spatial or temporal accuracy, and any required changes to the culture needed would require the creation of a new pattern. While 3D cell/Bio printing can print millions of cells and form large structures on a macroscale.

The Laser guidance system gives us the ability to place individual cells on an accurate micron level at very specific times without having to altering any existing cell cultures or systems. This means that we could use a 3D bio-printer to create a large cell culture, then with the laser guidance system add specific cells (e.g., epithelial) to observe how they react with the cell culture.

To perform any experiments with a laser guidance system, it requires a micron level accurate stage to actually pattern cells. Previously constructed optics and laser set-ups can then be used with the micron accurate stage to begin patterning cells. So, in order to further research on patterning cells, we have created a laser cell micropatterning system focusing its design on the need for accurate spatial and temporal results. This research will focus on the construction and design of the Joystick controlled 3-axis translational stage needed for patterning.

Lit Review

The idea of laser tweezers stems from a sequence of experiments conducted by Ashkin (Ashkin 1970, Ashkin and Dziedzic 1971, Ashkin 1992, Ashkin 1998). He showed that the radiation pressure from a focused laser beam could trap a micrometer sized, transparent, neutral particle (Ashkin 2000). This led to the naming of the two optical forces: one being scattering force which points in the direction of the incident light beam and the other being gradient force which points in the direction of the intensity

gradient of the beam (Ashkin 1997).The scattering force tends to move the particle to move along the beam propagation direction. The gradient attempts to move the particle in the direction towards highest intensity that is typically the focal point of the beam to form an optical trap.

A Gaussian beam is required for optical trapping because this beam gives a constant point of high intensity, where the particle is trapped, following a Gaussian curve. For a non-Gaussian beam with an average beam profile that looks like a Gaussian distribution, the particle in the focus region will experience a fluctuated optical force with a zero average trapping force.

The first biological experiments using a laser trap were conducted by Ashkin and Dziedzic (Ashkin and Dziedzic 1987, Ashkin, Dziedzic et al. 1987). They found that the tobacco mosaic viruses had absorbed too much heat from the laser (120mW green argon laser) and exceeded physiological constraints. An infrared NdYAG laser was then used, causing no harm to the living cells. This enabled them to move E.coli and trap yeast cells. Infrared light around 800 to 850 nm has a much longer wavelength then the visible light typical used in microscopy allowing researchers to use highly focused lasers on living cells without causing any lasting damage. Also, since cells and micro sized biological material is transparent (no light absorption), it allows us to use a higher intensity of around 150-200 mW. During laser-particle interaction, the photon momentum is transferred to the clear particles and therefore generate a strong optical force. Due to the strong focus, the gradient force is dominate to trap the particle. The photon energy in non-transparent materials is usually absorbed and turned to heat which kills cells. Many experiments have been conducted using optical trap techniques to move cells, organize cells, and even to measure the forces generated by cells (Lang and Block 2003). Optical tweezers help to discover the stepping motion of kinesin along a microtubule as well as RNA polymerase moving along DNA (Neuman and Block 2004).

These experiments lead to the practice of laser guidance-based cell micropatterning, which uses optical force to capture a cell similar to what can be done with an optical tweezer, then move it to a specific location on a variety of substrates. In principle, laser guidance works the same as optical tweezers, however it uses a different NA objective lens: laser guidance uses small NA objective, which typically has a working distance of a couple of millimeters rather than a working distance of several hundreds of microns in a laser tweezers, which uses a high NA objective. Laser guidance was introduced by Renn and Pastel in 1998 when they successfully suspended NaCl droplets(Renn and Pastel 1998). Later, Odde and Renn were able to use this system on embryonic chick spinal cord neurons, for the first time it was used on living cells (Odde and Renn 1999, Odde and Renn 2000). Laser guidance has a weakly focused laser that, unlike optical tweezers, is able to quickly move and pattern cells without dropping them. Using laser guidance with a beam waste of 3.6-4.2 microns and an accurate 3D stage, a neuronal network was accurately patterned as well as moving irregular rod-shape cardiomyocytes in an aligned manner (Ma, Pirlo et al. 2010, Pirlo, Ma et al. 2011).

In this report, I describe the design and construction of a motorized 3D stage to achieve laser guidance-based cell micropatterning.

Methodology

a. Power Box

In order to create a motor-controlled stage, a power source is required. The easiest way to power everything without using a premade function generator was to use an old Dell PC power supply.

The power supply has many wires on it however to power components such as Arduinos and motor drivers, 5V is needed which correlates to every Red wire. Red wires provide 5V, black wires provide ground and the green wire is a safety wire. To override the green wire safely, it was solderd to a

switch with a ground wire to turn the system on and off. For the hardware we are using, two red and two black wires (5V and ground) are needed to power everything in the circuit. Every other wire was cut short and capped for safety and to save space.

To encase the power supply and the circuits, a box was constructed to hold everything.

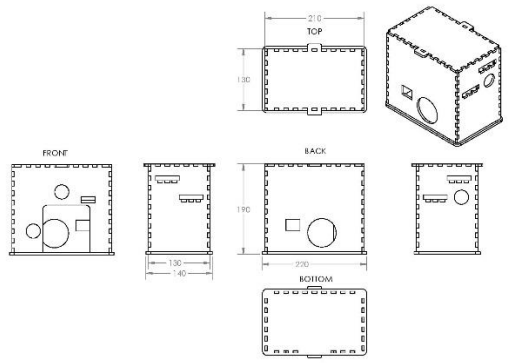


Figure 1: A Solidworks schematic showing the assembled pieces and what every side would look like. It includes minor measurements to show the total dimensions in mm.

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

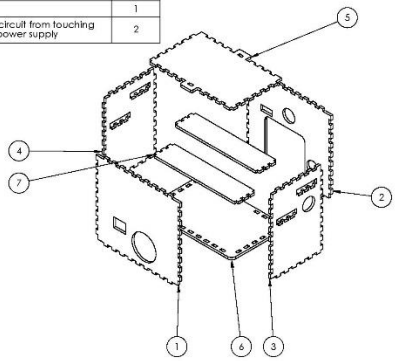


Figure 2: An Exploded view of the power box and all the parts labeled with short descriptions of the design

As seen in Figure 1 and 2, the box was designed in Solidworks has notches made into every side so that the pieces would fit together with flush corners. 5 mm Cast Acrylic was used and each part was cut using a laser cutting device. The notches are almost all 5mm tall and 10mm long to keep a relatively symmetrical pattern allowing the design to be copied more easily. Large vent holes (Figure 1: part 1 and 2), wire holes for the motor (part 3) and the controller (part 2), a hole for leftover power supply wires (part 2), a hole for the power switch (part 2) and a hole for the power cord (part 1) are all included in the design. The board holes (on parts 3 and 4) and the board holders (part 6) were made to hold the circuits on top of them so they do not touch the power supply or one another. Two are needed since there is an XY circuit and a Z circuit.

b. Controller

To control the motors of the stage, a controller was created with a couple of added features. The motors must be able to move at different speeds to effectively control the laser trap and important positions must be able to be stored and returned to at ease. To achieve this, two slide switches and four push buttons are included in the controller's design. To actually move the motors, two Joystiic v1.1 - Qwiic Joystick Breakout pieces from Sparkfun are used. Lastly four assorted standard LEDs are used for visual cues.

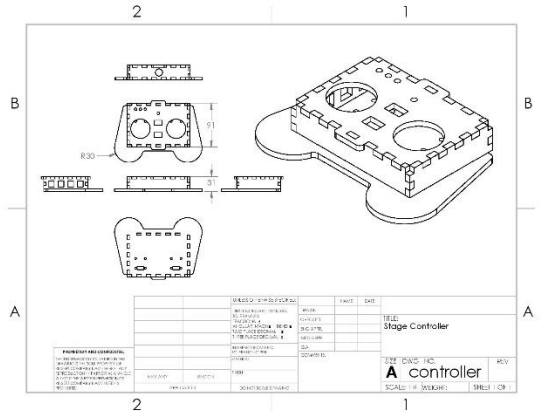
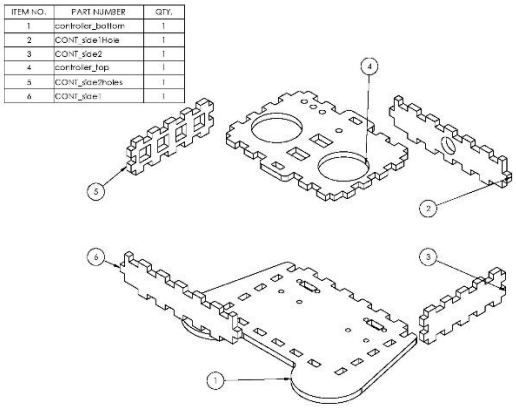


Figure 3: Solidoworks model showing the controller design and the sides. Includes basic mm dimensions to show the relative size of the controller.



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

Figure 4: An Exploded model of the controller listing the parts by number to be referenced.

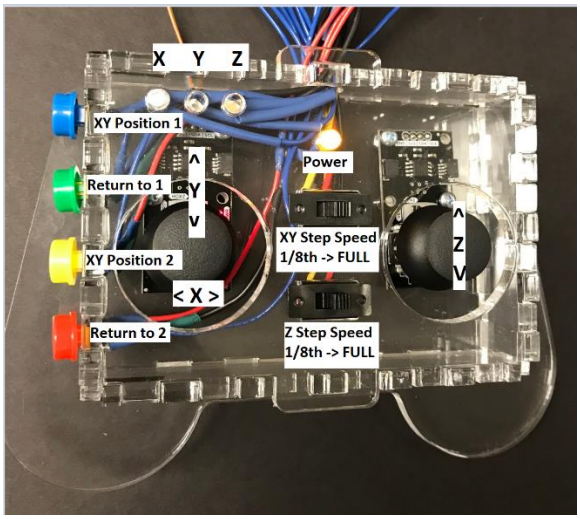


Figure 5: The assembled controller with all the components. The side four buttons (Store position 1 (Blue), Return position 1 (Green), Store Position 2 (Yellow), Return Position 2 (Red)), The two switches (Top = XY speed, left full step, right is eight step, Bottom = Z speed) and the four LEDs (middle = power light, Top left = X motor moving, Top middle = Y motor moving, Top right = Z motor moving) are all included. The left joy stick controls X (up/down) and Y (left/ right) while the right joystick controls Z (up/down). Pushing the joysticks down or moving the right one left/right are unused and could be coded to add more features.

The controller was designed with 5mm acrylic and has notches very similar to the power box. Laser cut holes were made to fit the LEDs, Buttons, Switches and the Joysticks. A wire hole is made to expel wires (part 2) and screw and wire holes (part 1) secure the Joysticks in place while giving easy access to their separate wires. The bottom piece (part 1) has two extruding parts to fit comfortably in one's hand and the top piece (part 4) has handles so the controller can be taken apart and repaired.

c. Stage design

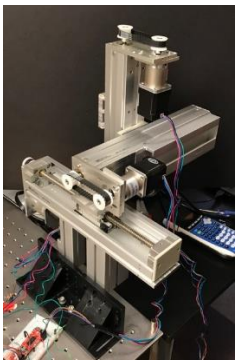


Figure 6: The stage used with the new motors (Nema 17) attached with their corresponding pulleys and timing belts

A previously built 3 axis translational stage used as the base for the project. To provide more accuracy in the realm of microns, the original stepper motors were replaced by Three Nema 17 Planetary Geared Stepper Motors with a 51:1 gear reduction ratio. This causes the original step of the motor to be a 50^{th} in step size. Pulleys were needed as well, so 6 pulleys (XL series lightweight timing

belt pulley, 1” OD, 12 Teeth, .2” pitch) and 3 timing belts (XL series timing belt, width 3/8”, 8” circumference, 40 teeth, .2” pitch) were ordered off McMaster-Carr. To fit our Nema 17 motors and the stages shafts, the 3/16” shaft diameters of the pulleys were machined by the Clemson University machine shop to 8mm (3x) and 5mm (3x).

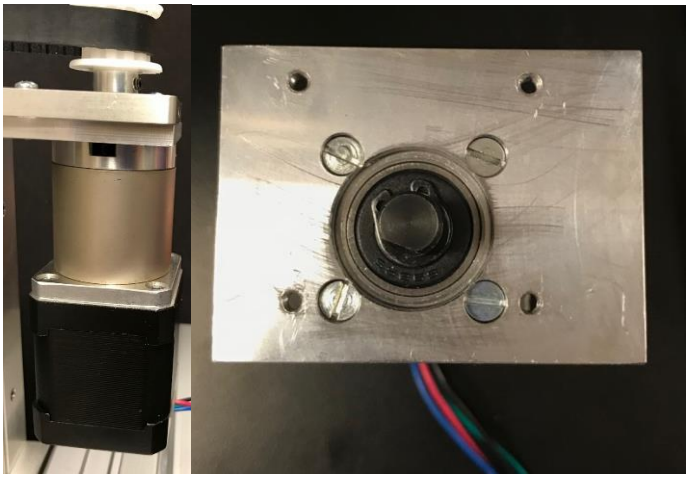


Figure 7: (left) the Nema 17 motor connected to the stage with the pully fastened to the shaft. (right) Aluminum plate with counterbored holes attached to the motor, used to attach the motors to the stage with threaded holes.

To fit the new motors to the system, aluminum rectangles were machined using the Clemson University Machine shop. Four counterbored holes align with the motors gear box and fasten the aluminum plate to the top of the motor with M3 x 3 screws. Four other holes were tapped so that M3x12 screws could be placed through the top of the stage and secured to the aluminum plate. This way the plate is adjustable allowing us to tighten or loosen the timing belt. All three motors (XYZ) are identical.

d. Hardware

All the components of the controller, plus the motors, must be connected and controlled by a driver or Arduino. Two Arduino Nanos were used along with three Stepper Motor Easy drivers from Sparkfun. Both Joysticks require an i2C communication and the Nano has a limited amount of ports which is why two Arduinos are used. On the Arduino A4 port is SDA and the A5 port is SCL used for i2C, while A0-A3 can also be written as D14-D17 giving us more digital ports. The Red wires (5V) from the power supply power the separate Arduino circuits, called XY circuit and Z circuit. This is the reason there are two board holders in the power box.

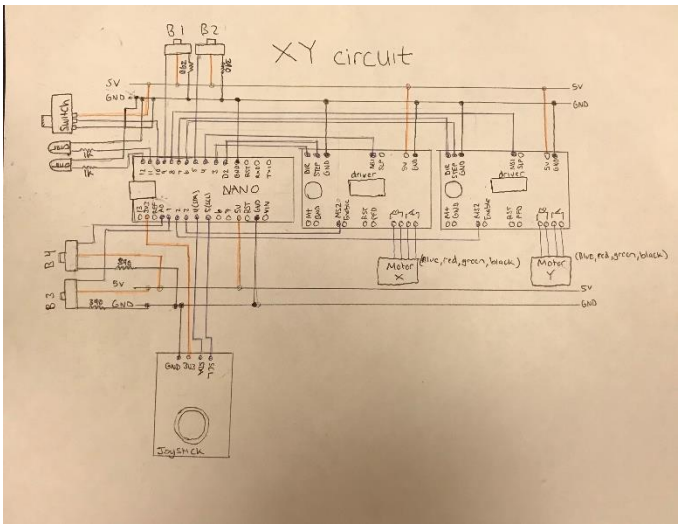


Figure 8: Circuit Schematic of the XY Circuit containing all the proper connections and parts attached to it. This circuit has two LEDS, four buttons, one switch, one joystick, and two motors. The Arduino drives each motor separately with the help of two easy drivers.

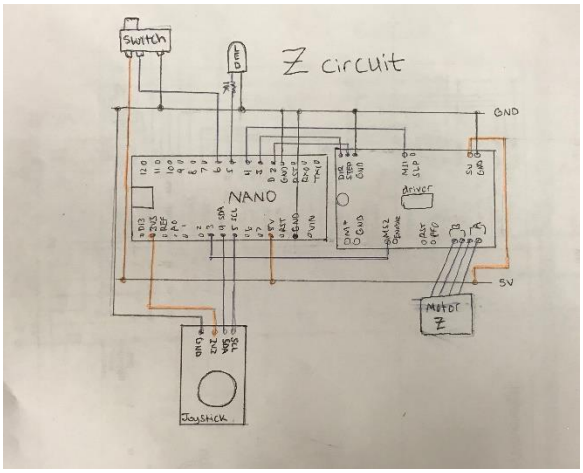


Figure 9: Circuit Schematic for the Z Circuit. This contains one Arduino and one easy driver, along with one Joystick, switch, LED and motor.

The Circuit Schematics as seen in Figures 8 and 9 show all the necessary connections made to make everything work properly mechanically. It must be noted that the LEDs all contain a 1K Ohm resistor each, and the Buttons all contain a 390 Ohms resistor, soldered into the circuit. Also, the Joysticks require a 3V3 instead of 5V so they had to be connected to the Arduino 3V3 port instead of the long 5V line provided by the power supply.

e. Software

Using the Arduino software interface (similar to C programming), the software addresses the correct pinout given by the circuit. The code for XY is able to keep track of the number of steps taken by the motors staring from zero each time it is turned on. There are no limits set by the code, so the point the motors are in when turned on are considered the origin. When a position is stored by pressing a button (1 or 3), it displays the position on the screen, then when a return button is pressed (2 or 4), the

screen shows the individual steps of X then Y. It must be noted that positions only work for the XY direction not the Z direction which must be moved manually each time. Changing the switch will causes the step number to either be 8 per step (full step, fastest speed) or 1 (1/8th step, slowest speed) which is why the position numbers can change so rapidly. The code will step the correct amount of times regardless of switch speed. Switch speed can also be manually changed within the code by changing the HIGH or LOW output of the MS1 and MS2 ports on the Easy drivers. So you can also use ½ step (4 steps) and ¼ step (2 steps). Lastly, the Joystick reads a range and based on that the motors move counter clockwise or clockwise depending on the position of the joystick (up/right = Clockwise, down/left = Counterclockwise).

The code in Z direction does not track position but it is identical in motor stepping, switch speed, and Joystick code.

Results

The motors can accurately move clockwise or counterclockwise when given input from the controller's joysticks. X, Y, and Z LEDs light up with the corresponding direction in order to give easy visual cues since the motor movement may be very small and hard to see. By hitting the switch, the speed of the motors turns is dramatically altered, as a 1/8th step (low speed) is visually hard to see movement at all. Full steps can be seen moving rather quickly, but it is still a very small and slow motion due to the motors gear reduction.

The two store position buttons can easily store the value of steps taken and keep it until they are pressed or the system is turned off and the value is replaced. When the return position buttons are pressed the motors move back to the stored position and if the stored positions are different you can shuffle between them by simply pressing both return buttons.

Figure 10: Two distinct positions on the slide that were stored with the store buttons on the controller. The two return buttons were pressed multiple times and were able to produce the same image each time.

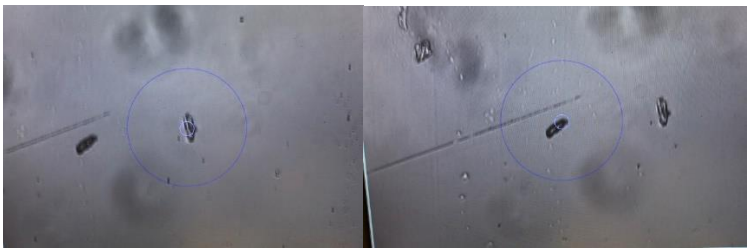


Figure 11: Two new distinct positions toggled back and forth using the two return buttons.

Using a USB camera and a white LED (given 5V and a 300 Ohm resistance) we created our own imaging system. The Camera was fastened to image through an objective lens that looked onto a petri dish with marker spots and hair on it. The Stage was able to store two positions and toggle between the

two spots accurately as seen in Figure 10. The joysticks could easily maneuver over the slide and the stored positions could be changed and returned to at any point. Figure 11 shows an image of random particles in tap water that were imaged using the previously set up laser guidance system. The imaging system is connected to the laser used for guidance so cell patterning experiments can be done with the new stage very soon.

Discussion

With the current set up, the stage can be used with laser guidance and successfully toggle between positions and move around. This gives us a step closer to having very accurate spatial and temporal resolution which is vital to cell patterning with laser guidance. The current system could be used as a laser guidance stage that could potentially pattern cells.

However, the speed of the movements is a major issue. With the time constraints, the software was made hastily and the step speed system does not allow for a wide range of speeds to choose from. Future work would have to be made in speeding up the motors so positions can be toggled between much faster than they can currently. A quicker speed fix would be to make the two motors run together when returning to positions, rather than running X then Y.

Also, using two Arduinos and having the hardware rest in the power box must also be improved. Two Arduino Nanos were used to obtain more digital ports and to easily use two separate i2C slaves (Joysticks). The joysticks conflicted each other when connected to the same Arduino so due to time constraints two were used.

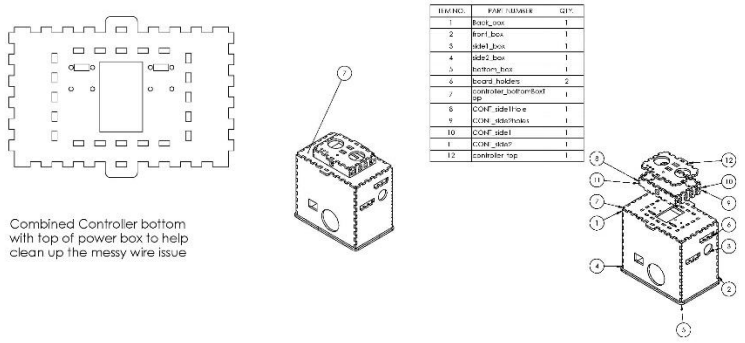


Figure 12: A Solidworks schematic shows the integration of the power box and the controller. The top piece of the box and the bottom of the controller were combined to allow for all the wires to neatly organize inside the box. This would solve any issues with messy wires and wires that restrain the controller from moving very far from the box

Placing the controller as an attachment to the box cleans up the wires and is also temporary, as the controller pieces can be separated from the box and placed as a controller and box again. To further condense the system, we would need to combine the Arduinos by learning to use both joysticks on it. This would also allow for the Z direction to be included in the XY store/return buttons.

Conclusion

The Joystick controlled stage is able to achieve an accurate spatial and temporal movement needed to perform laser guidance. The buttons effectively return to the stored positions and the controller is easy to use when navigating slides and cell cultures.

With the newly made system, we can use it with our pre-existing laser guidance system and begin to perform biological research with it. We will be able to pattern cells, such as epithelial cells, on cell cultures printed from our 3D Bioprinter. The stage will allow for us to trap cells and place them quickly and effectively to create a newly patterned cell culture that can mimic in vivo interactions. We should expect to see the laser patterning of cells help to improve the Bio printed pattern by adding accurately spaced cells within a system that is only relatively accurate.

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