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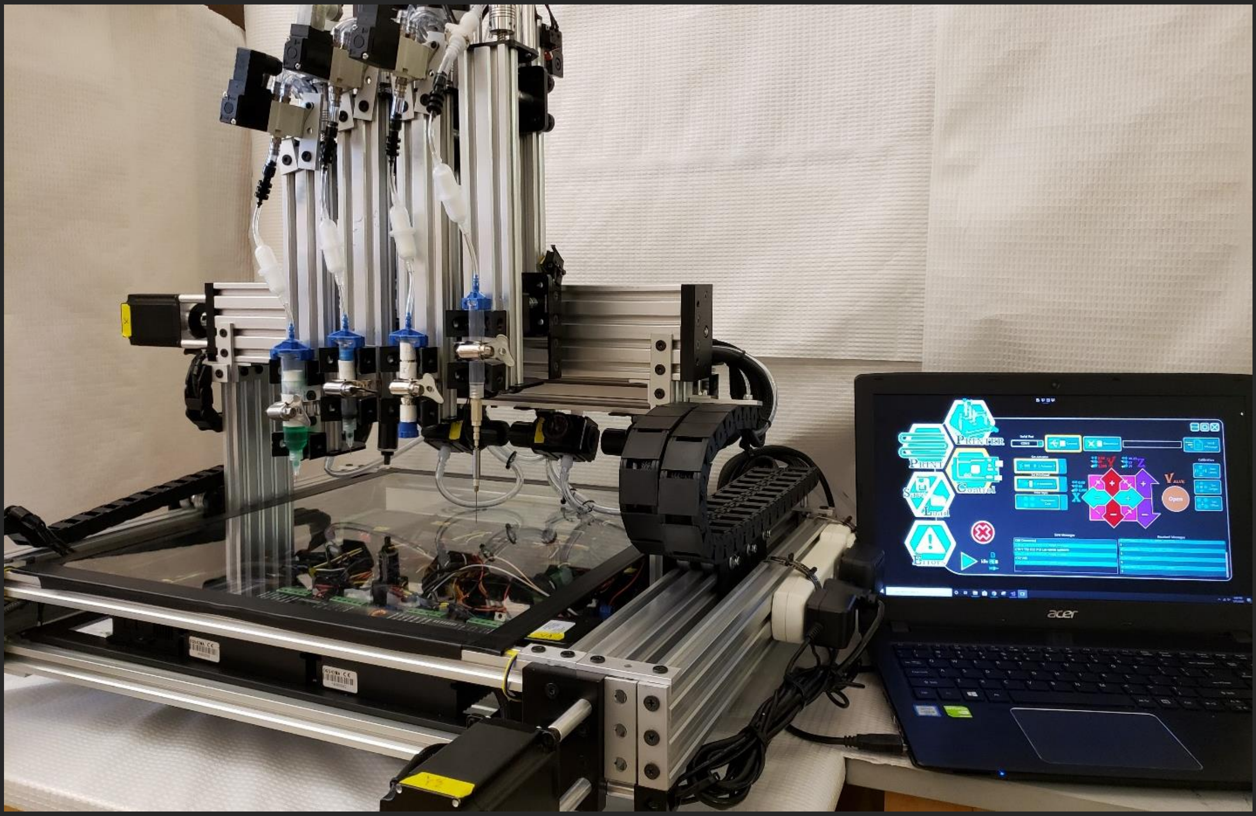
Introduction

This is the complete guide to ModiPrint, a partially open source bioprinter. Construction and operation of this bioprinter requires the user to:

1. Acquire all hardware via other vendors.
2. Assemble the hardware using this guide.
3. Install the open source desktop software.
4. Purchase the firmware from ModiPrint's website.

This document covers the entire process of building a bioprinter, navigating its software, preparing bioinks, and optimizing prints.

It is recommended that users read this guide sequentially from beginning to end. This document should be read digitally to take advantage of hyperlinked text (example). The entire documentation (with all supplemental files and folders) should be downloaded and file locations unchanged for hyperlinks to function correctly.



QuickStart Guide

To set up the bioprinter:

1. Acquire parts for and assemble the bioprinter.
2. In the desktop program, set printer settings.
3. In the desktop program, save your parameters.

To perform any print operation:

1. In the desktop program, load your previously set printer parameters.
2. In the desktop program, calibrate the machine with the "Find Limits" and "Set Origin" buttons. Use of multiple printheads will require calibrating printhead offsets.
3. Generate a g-code file either by hand in a text editor or via a slicing program.
4. In the desktop program, set the appropriate material settings such as print speeds and styles. Then upload the .gcode file.
5. Connect to the microcontroller and maneuver the intended printhead to the starting position(origin) of the print.
6. Load the material.
7. In the desktop program, begin the print sequence.

Bioprinter Assembly

This section of the guide will example the replication of the hardware as seen in this publication TO DO. This process was designed to be simple with a hardware suite consisting entirely of commercially available parts. The assembly process is guided by CAD files, wiring diagrams, and hyperlinked parts lists. While not much skill is required to replicate the machinery, there is a large time commitment of 100-200 hours.

Determining Printer's Specs

ModiPrint is designed with some modularity to fit the needs of individual labs. Therefore, a few things must be decided before the assembly process.

XYZ Stage Size

For sterility, ModiPrint is intended to be small enough to fit into most laminar flow hoods. If the size of the device is of no concern, then skip this section.

The default hardware's dimensions of this guide come out just under 700 mm x 700 mm x 700 mm. Most laminar flow hoods have the vertical and horizontal clearance to fit the device, but the Y actuator motors may need to pop out underneath the safety screen (pictured right).

The profile of the machine can be made more compact by cutting the length of the V-slot beams and Lead Screws. This will reduce the range of the XYZ stage and potentially reduce the number of printheads that can be mounted. The hardware assembly section contains hyperlinks to CAD drawings of the hardware and it is always recommended that modified designs be modeled in CAD as heavy modification may not physically fit.

Number and Types of Printheads

Before assembling the hardware, decide the number and type of printheads to build. ModiPrint supports the use of multiple printheads each mounted on independent Z actuators. ModiPrint's software supports the use of binary valve-based printheads and stepper motor driven printheads. Both types of printheads can be programmed for droplet printing with or without concentration gradients, and extrusion printing.

This hardware guide details the design of pneumatic extruders and microdispensing inkjets. While motor-driven printheads are supported by the software, the hardware guide does not make any recommendations for its parts and assembly. There are many open source motor-driven printhead designs available from other sources. The only requirement is that the motor of this printhead be controller by a stepper driver with step and direction inputs.

Pneumatic Extruder Printhead: Features a syringe barrel as a material reservoir with a Luer lock to attach a variety of nozzle sizes. The operation is controlled via a solenoid valve where the material is printed with pressure and withheld from dispensing via vacuum. A syringe piston regulates the application of pressure forces. It is recommended that this printhead can serve as a general-purpose extruder of fluids of various viscosities.

Microdispensing Inkjet Printhead: Features a fast-acting solenoid valve downstream of a material reservoir intended for dispensing low viscosity droplets as small as the nanoliter range. It is recommended that this printhead be used for droplet printing and generating small molecule concentration gradients.

Motor-Driven Printhead: Note that while motor-driven printheads are supported by the software, the hardware guide does not make any recommendations for its parts and assembly. There are many open source motor-driven printhead designs available from other sources. ModiPrint's use of 20 mm V-slots is very capable of mounting these designs to ModiPrint's Z actuators.

Motor-driven printheads are recommended for dispensing low viscosity materials. Low viscosity materials have uncontrollably high dispense rates when used with pressure driven dispensers. Motor-driven printheads directly control dispense rates and are therefore suited for these materials.

Number of Printheads: The default hardware specified in this guide can fit 5 printheads. The number of printheads can exceed 5 by lengthening the X actuator by replacing the C-Beam 20x80mm Linear Rail and 8mm Lead Screw with other commercially available, longer variants. Note that this will increase the width of the system. Note that not all printheads can reach all areas of the print surface (the rightmost printhead cannot traverse to the leftmost areas of the print surface).

Custom Modifications

While the designs in this guide are specific, the designs also encourage user modification. The frame and all actuators are built with very modular 20 mm V-slot components from Openbuilds that fit M5 screws and nut blocks. Therefore, attaching hardware to any part of the system is relatively simple. If heavy modification is required, feel free to contact us with ideas.

Hardware Assembly

If these diagrams and models seem daunting, don't worry! With all the parts in hand, assembly and wiring will be intuitive.

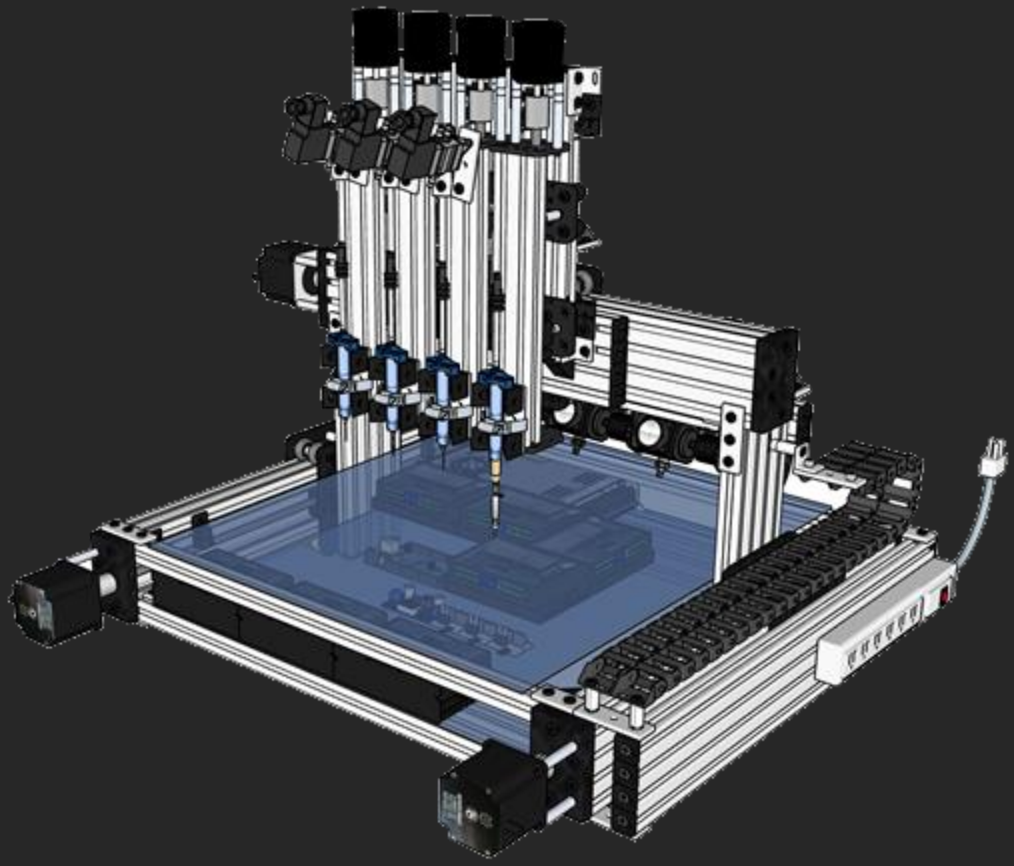
Before proceeding with this section, determine the specifications of the printer. Each section of the assembly guide will detail the parts list, assembly instructions, wiring diagrams and special considerations for different sections of the bioprinter.

Parts Lists: The parts lists is an Excel file included with the documentation. It will contain the name of all parts as well as how many are required, links for where to purchase these parts, and the price of each part as of 2019 (shipping and taxes not included). If ever in doubt about the use of a part, be sure to consult the part's manual that is provided by the manufacturer.

When first opening the spreadsheet, enable macros when prompted. The only cells that should be edited are in the top left with turquoise backgrounds. Input the total number of each printhead to those tabs and refresh (Alt + F5) the sheet to update the "Parts Total" section. The "Parts Total" section will display the total number of parts and price required for a complete build of the bioprinter. Clicking on any underlined cells will navigate to the online parts store for that part. The other sections of the parts list subdivides the parts total to more manageable sections.

Sketchup Model: The spatial arrangement of these parts is detailed in the Sketchup (.skp) files included with the documentation. These files can be viewed for free with Sketchup Make 2017. To see how parts are assembled, it is recommended that users dismantle the bioprinter in Sketchup. To move the camera in Sketchup, hold down the mouse wheel or rotate the mouse wheel. To move a part, press M, left click on a part, then drag the part to a desired location. Initially, the Sketchup file of the bioprinter will have related parts grouped together. To ungroup these parts, right click on the group and select "Explode". For more advanced actions, see Sketchup's tutorial.

Below is an image of the Sketchup model of the bioprinter in the McCloskey lab. This can be found in the "Bioprinter" Sketchup file that is included with the documentation.



Electrical Wiring: Due to technical limitations, wires in this system are not represented in the Sketchup file. Therefore, the wiring diagrams in this guide will visually detail the electronic wiring separately from the Sketchup 3D models.

Wiring will be one of the messiest endeavors in this project. It is also the easiest area for error. Therefore, it is crucial to follow best practices for organization and long-term durability.

There are no wiring products that make this wiring process completely plug-and-play. This guide recommends the purchase of single strands of wire where junctions will need to spliced, soldered, and covered in heat shrink. Most stripped ends of basic wires will slot into the drivers via screw terminals whereas wires connected directly to the microcontroller will need to be jumper wires. It's always recommended that users lightly tug on finished splices and connections to ensure durability and inspect

Keep in mind that some electronic components will move with the XYZ stage. Included in the parts list and Sketchup models are drag chains that hold the wires in safe locations as parts move. These wires will also run along the sides of and through the frames and actuators. Therefore, the parts list also includes V-slot covers which tucks wiring neatly within the crevices of the frame.

Finally, keep the wiring organized. All wires should be color-coded such that the purpose of each wire is known at a glance. The wiring diagrams in this guide example a coloring system where 5V microcontroller power are all red, outlet power sources are all white, grounds are all black, etc. It is also important to group relevant wires together in cable sleeves (it's in the parts list). For the example of an actuator, a single sleeve groups all four coil wires of the stepper motor, two wires of the limit switch, and two wires of the solenoid valve. Both ends of the cable sleeve are covered in heat shrink and this collectively protects the wires as they travel through the drag chains. TO DO picture of cable stuff

DQ542MA Stepper Driver: Each actuator will be controlled by one of these DQ542MA drivers. Each of these drivers has a microstep setting that affects the torque and resolution of the actuators. 1/8th microstepping is the recommended setting for ModiPrint hardwareBelow is an image showing the pins on the DQ542MA stepper driver that sets microsteps to 1/8th.

Understanding the following details is only necessary if deviating from recommended hardware. To change to another microstep settings, toggle pins 5-8 according to this table:

Microstepping	Pin 5	Pin 6	Pin 7	Pin 8
1	OFF	ON	ON	ON
1/2	ON	OFF	ON	ON
1/4	OFF	OFF	ON	ON
1/8	ON	ON	OFF	ON
1/16	OFF	ON	OFF	ON
1/32	ON	OFF	OFF	ON
1/64	OFF	OFF	OFF	ON

Finer microstepping will result in finer actuator resolution but lower torque. Lower torque equates to lower acceleration, which results to slower prints and less consistent filament diameters. The formula to calculate actuator resolution is below:

$$Distance\ per\ Step = \frac{Screw\ Pitch * Stepper\ Driver\ Microstepping}{Motor\ Steps\ per\ Revolution}$$

With this guide's recommended 400 step/rev XY stepper motor, 8 mm screw, and 1/8th microstep setting, the distance per step is 5 μm.

Pneumatics: Like the electrical wiring, the pneumatics is mostly DIY and will similarly run through drag chains. Unlike the electrical wires, tubing in this application is less numerous. Therefore, organization is more disinteresting. However, tubing is less flexible, and for compactness, tubing should be cut close to the minimum required size for connection. This guide will semi-arbitrarily recommend 3/16-inch ID tubing for most of the connections. While tubing diameters can be modified, changing this parameter necessitates changing all fittings to match.

Y Actuator & Frame

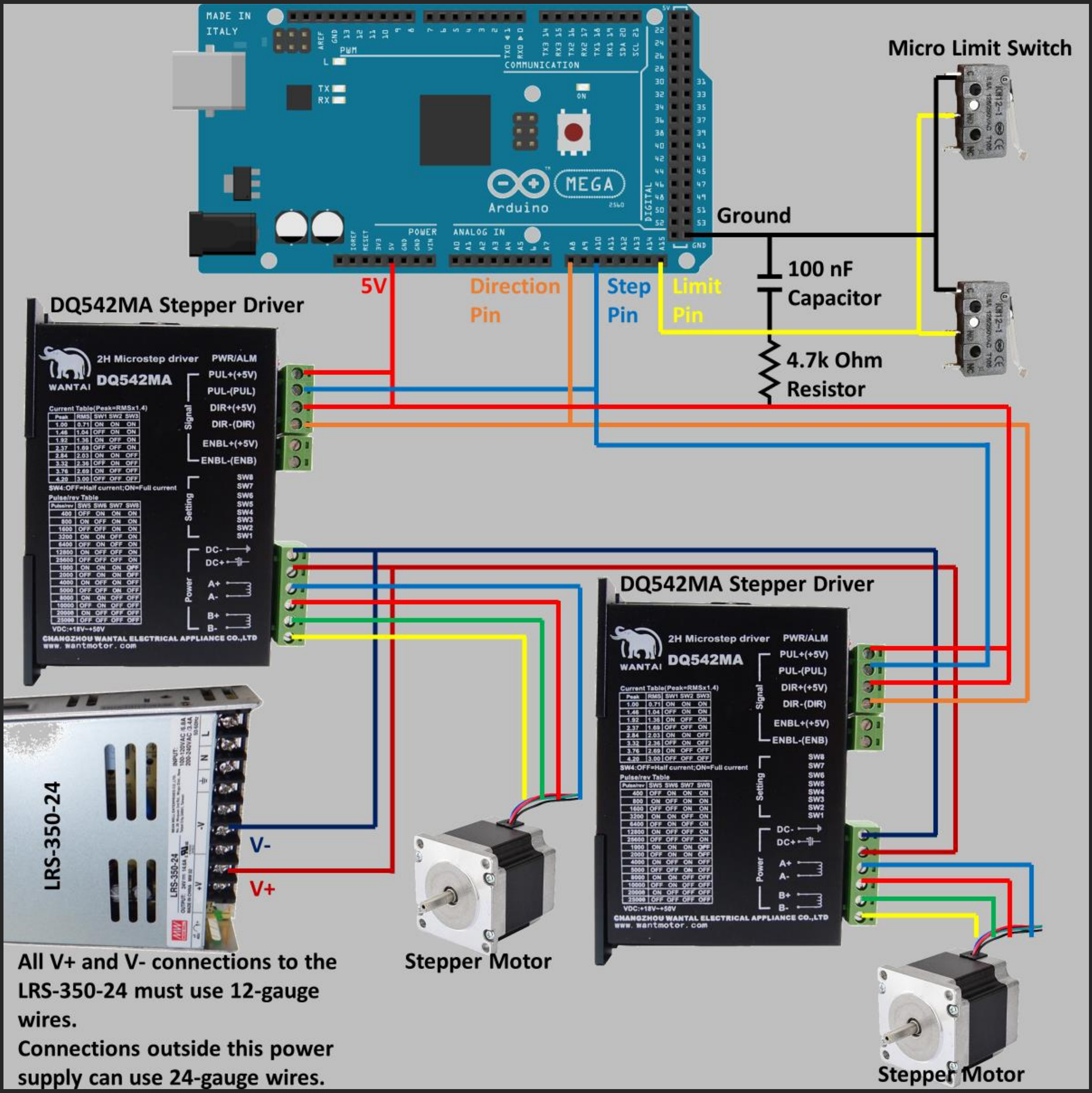
This is necessarily the first section to assemble. It consists of the base and frame of the bioprinter which is integrated with the 2 Y actuators. It also contains the platform for the electronics and the support structures for which the X actuator will be mounted.

For an example part arrangement of the entire bioprinter, open the "Bioprinter" Sketchup file.

Y Actuators: On the parts list, scroll down to the "2 Y Actuators" section to see which parts are required. Open the "2 Y Actuators" Sketchup file for part arrangements. Things to look out for:

- The anti-backlash nut needs to be tightened to the threaded rod.
- The eccentric spacers on the wheel assembly need to be adjusted to tighten the wheels to the c-beam.
- It's easier to mount the limit switches after wiring them.

The wiring for the Y actuators is as follows:



The step, direction, and limit pin connections to the microcontroller can be connected to any GPIO pin. The ID for the GPIO pin will be physically marked on the microcontroller. Be sure to set the appropriate Pin ID values for each of the three pins under "XYZ Stage Settings" in ModiPrint's desktop program.

Frame & Print Surface: On the parts list, scroll down to the "Frame & Print Surface" section to see which parts are required. Open the "Frame & Print Surface" Sketchup file for part arrangements. Things to look out for:

- The 2 Y actuators need to be assembled first as they are a part of the structural frame of the bioprinter.
- The print surface can be any material sheet of 440 x 500 mm and needs to be rigid. Glass is recommended as many hardware stores offer it cut to size and because it's aesthetic to see all

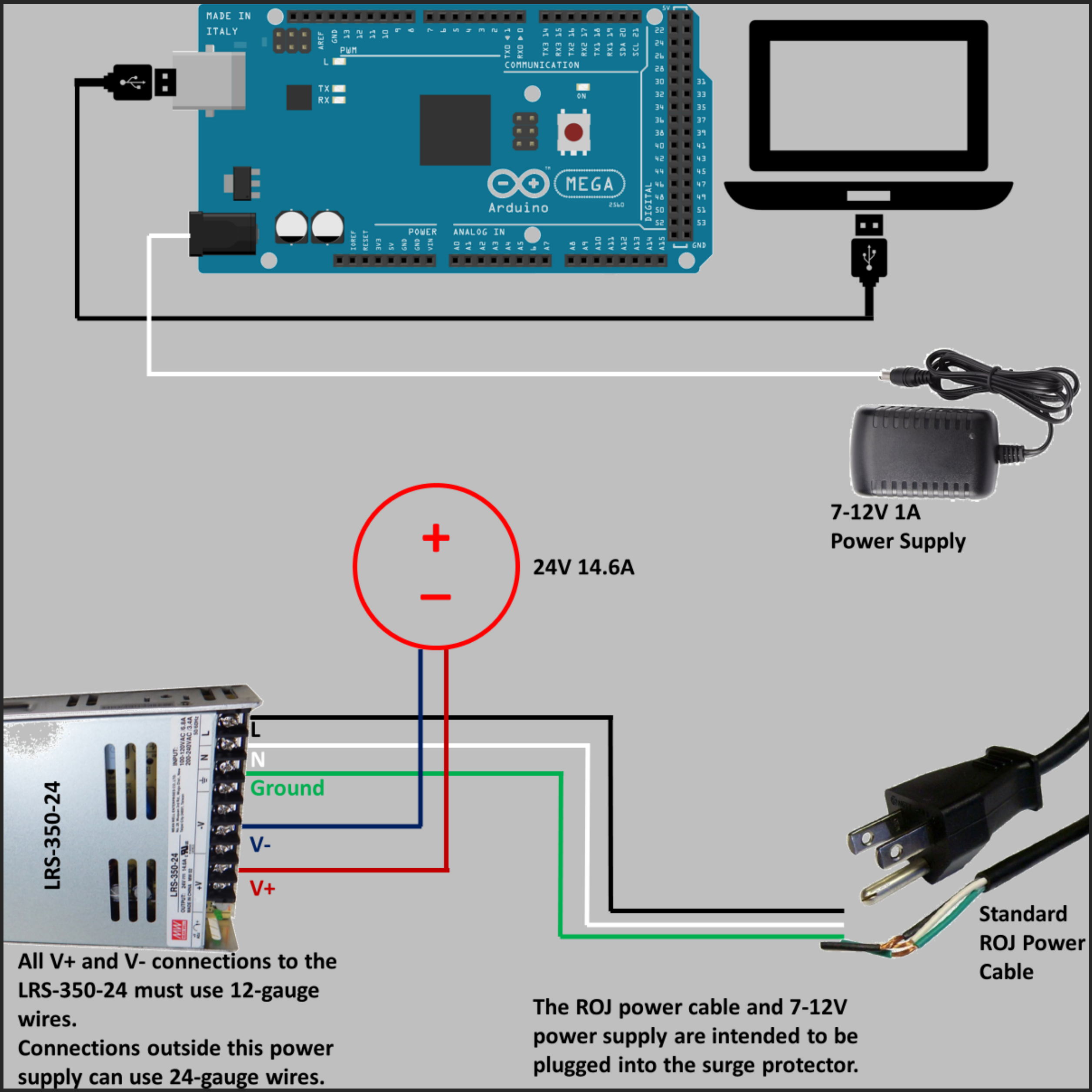
the electronics underneath. The bottom surface (where the electronics are placed) also needs to be 440 x 500 mm except it does not need to be as rigid. Plexiglass is recommended for the bottom surface for similar ease of access and increased durability over glass.

- The electronic drivers in the Sketchup model are only there to demonstrate an example placement that may help wire management. Electronics placement is arbitrary as long as wires are connected correctly, and wiring is organized.
- The print and bottom surfaces can be secured any number of ways. Moderately strong tape (such as electrical tape) is stable enough not to affect the precision of prints. TO DO picture

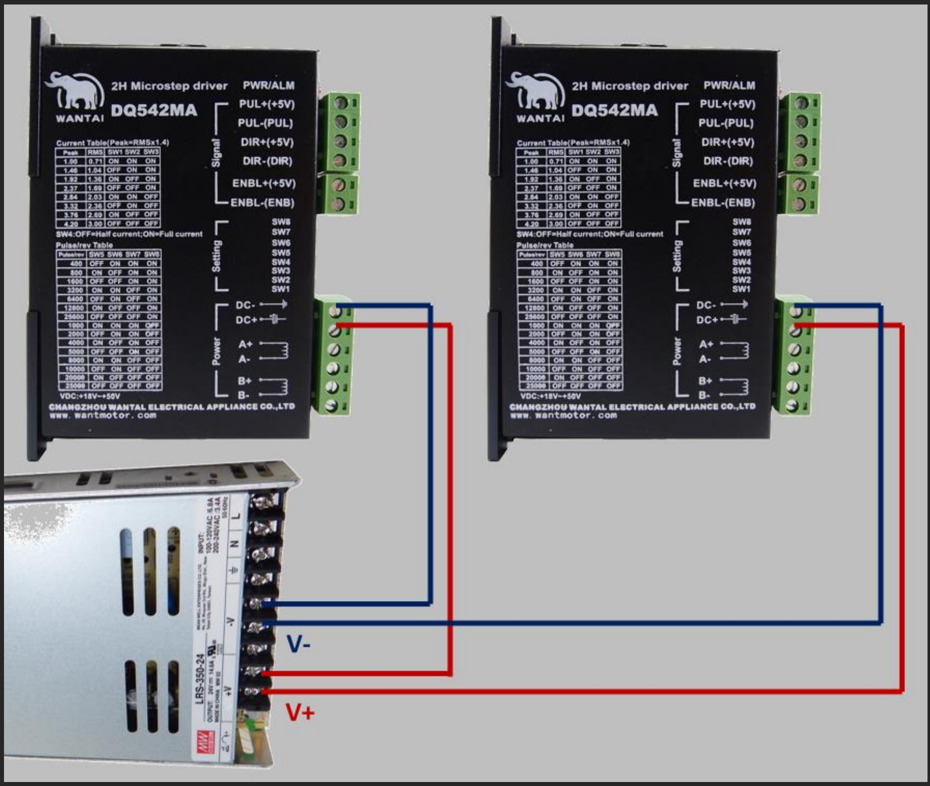
Y Drag Chain & Outlet Extension: On the parts list, scroll down to the "Y Drag Chain & Outlet Extension" section to see which parts are required. Open the "Y Drag Chain & Outlet Extension" Sketchup file for part arrangements. Things to look out for:

- The 2 Y actuators from the previous section need to be assembled first as they are a part of the structural frame that this extension is attached to.
- The drag chain lengths must be adjusted. A drag chain that is too short would not accommodate the full length of movement of the Y actuators. A drag chain that is too long will extend beyond the dimensions of the bioprinter.
- The surge protector can be secured any number of ways, all of which are bootstrappy. Below is an example with hose clamps. Some sturdy tape would also work.

Power & Microcontroller: The power supplies and microcontroller are wired as such:



Be mindful that wires only hold so much current. It's advised that each separate output of power uses separate wires. For example, two sets of power wires should supply two different drivers as such:



X Actuator & Mounts

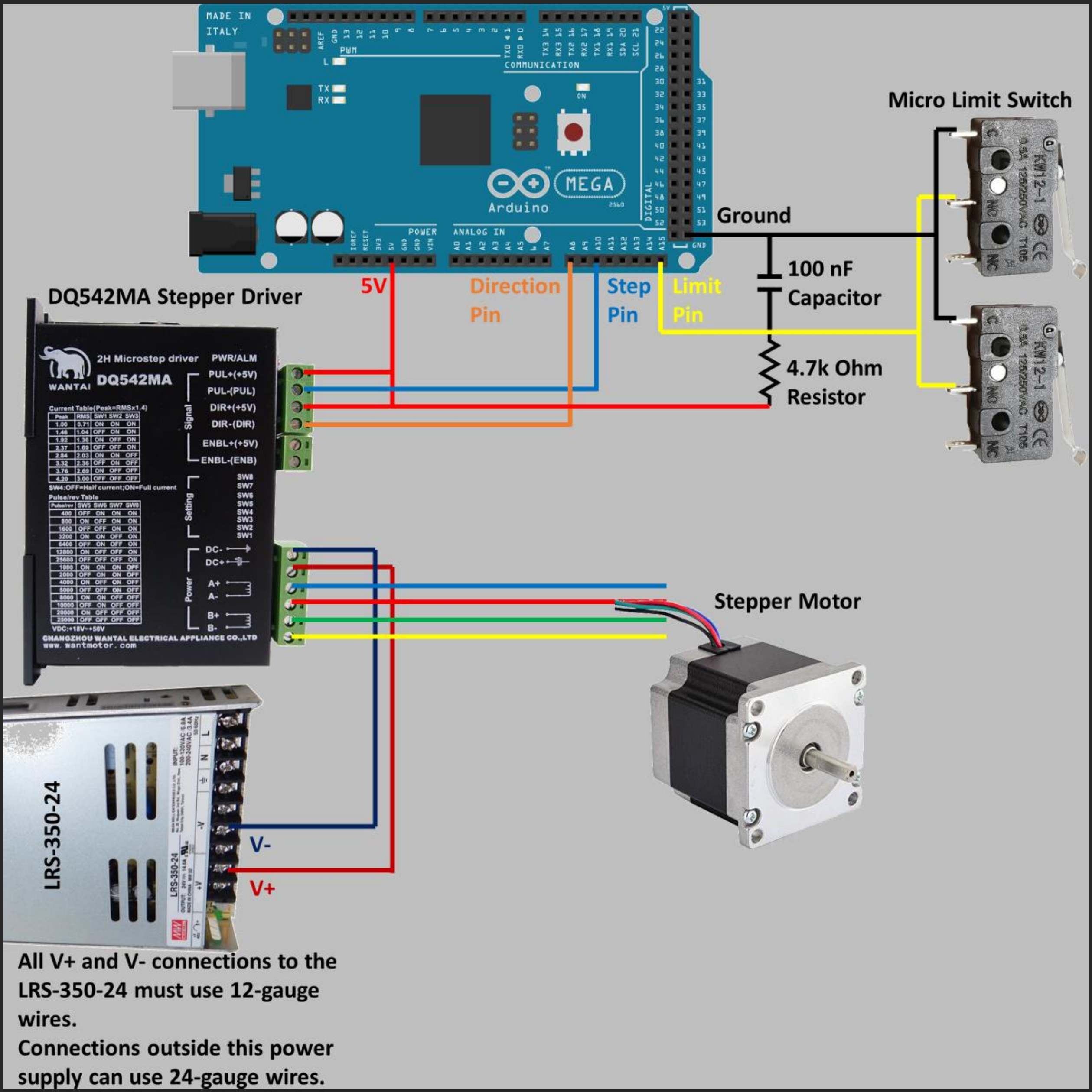
This is the X actuator and supporting structural component for the printheads and pneumatics. It is mounted on supporting beams of the Y actuators which need to be assembled before this.

For an example part arrangement of the entire bioprinter, open the "Bioprinter" Sketchup file.

X Actuator & Printhead Mount: On the parts list, scroll down to the "X Actuator & Printhead Mount" section to see which parts are required. Open the "X Actuator & Printhead Mount" Sketchup file for part arrangements. Things to look out for:

- The 2 Y actuators from the previous section need to be assembled first as they are a part of the structural frame that this extension is attached to.
- The anti-backlash nut needs to be tightened to the threaded rod.
- The eccentric spacers on the wheel assembly need to be adjusted to tighten the wheels to the c-beam.
- It's easier to mount the limit switches after wiring them.

The wiring for the X actuator is as follows:



Wires from the X actuator should run through the drag chain attached to the Y actuators to the bottom surface where the electronics are housed.

The step, direction, and limit pin connections to the microcontroller can be connected to any GPIO pin. The ID for the GPIO pin will be physically marked on the microcontroller. Be sure to set the appropriate Pin ID values for each of the three pins under "XYZ Stage Settings" in ModiPrint's desktop program.

X Drag Chain & Pneumatics Panel: On the parts list, scroll down to the "X Drag Chain & Pneumatics Panel" section to see which parts are required. Open the "X Drag Chain & Pneumatics Panel" Sketchup file for part arrangements. Things to look out for:

- The 2 Y actuators from the previous section need to be assembled first as they are a part of the structural frame that this extension is attached to.

- The drag chain lengths must be adjusted. A drag chain that is too short would not accommodate the full length of movement of the X actuator. A drag chain that is too long will extend beyond the dimensions of the bioprinter.
- Represented in the Sketchup file are multiple pressure regulators. The number of regulators required will use varies depending on the number of pneumatically driven printheads (pneumatic extruder or microdispensing inkjet) to be installed. All pneumatically driven printheads can be connected to a single pressure regulator or, if individual pressure control is required, each printhead can be connected to individual pressure regulators.

Z Actuator & Printheads

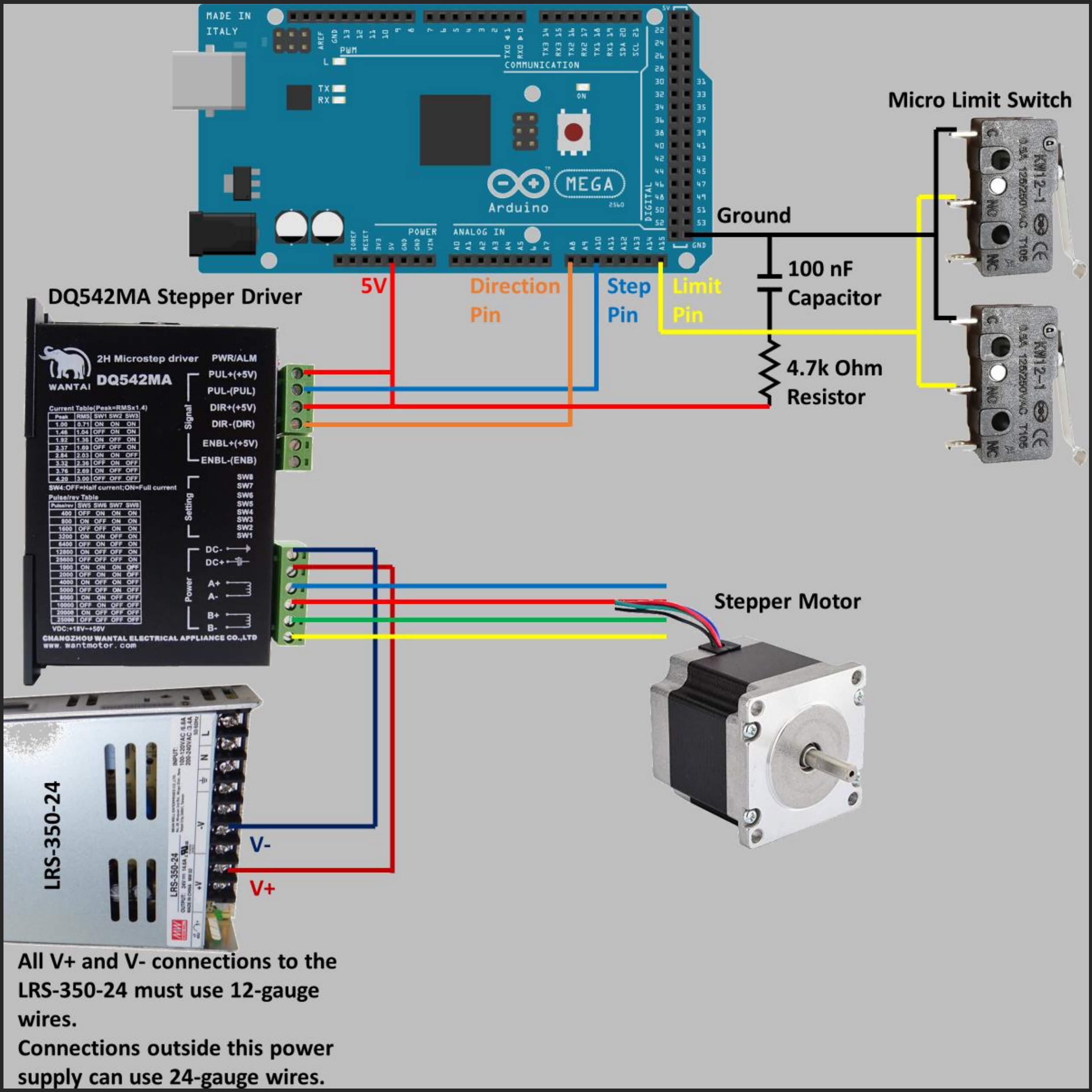
Z actuators and printheads and are mounted on a printhead mount attached to the X actuator which needs to be assembled first. Each printhead is intended to be mounted on its own Z actuator although the software can support modified arrangements with multiple printheads on a single Z actuator.

For an example part arrangement of the entire bioprinter, open the "Bioprinter" Sketchup file.

Z Actuator: On the parts list, scroll down to the "Z Actuator" section to see which parts are required. Open the "Z Actuator" Sketchup file for part arrangements. Things to look out for:

- The X actuator from the previous section needs to be assembled first as they are a part of the structural frame that Z actuators are mounted on.
- The anti-backlash nut needs to be tightened to the threaded rod.
- The eccentric spacers on the wheel assembly need to be adjusted to tighten the wheels to the c-beam.
- One of the mini V gantry plates must have two of its holes widened by a 5-6 mm drill bit. The specific locations are detailed in the Sketchup file.
- It's easier to mount the limit switches after wiring them.
- The Z Actuator is the most likely part to be improperly built. Be sure to tighten and align all parts carefully.

The wiring for the Z actuator is as follows:



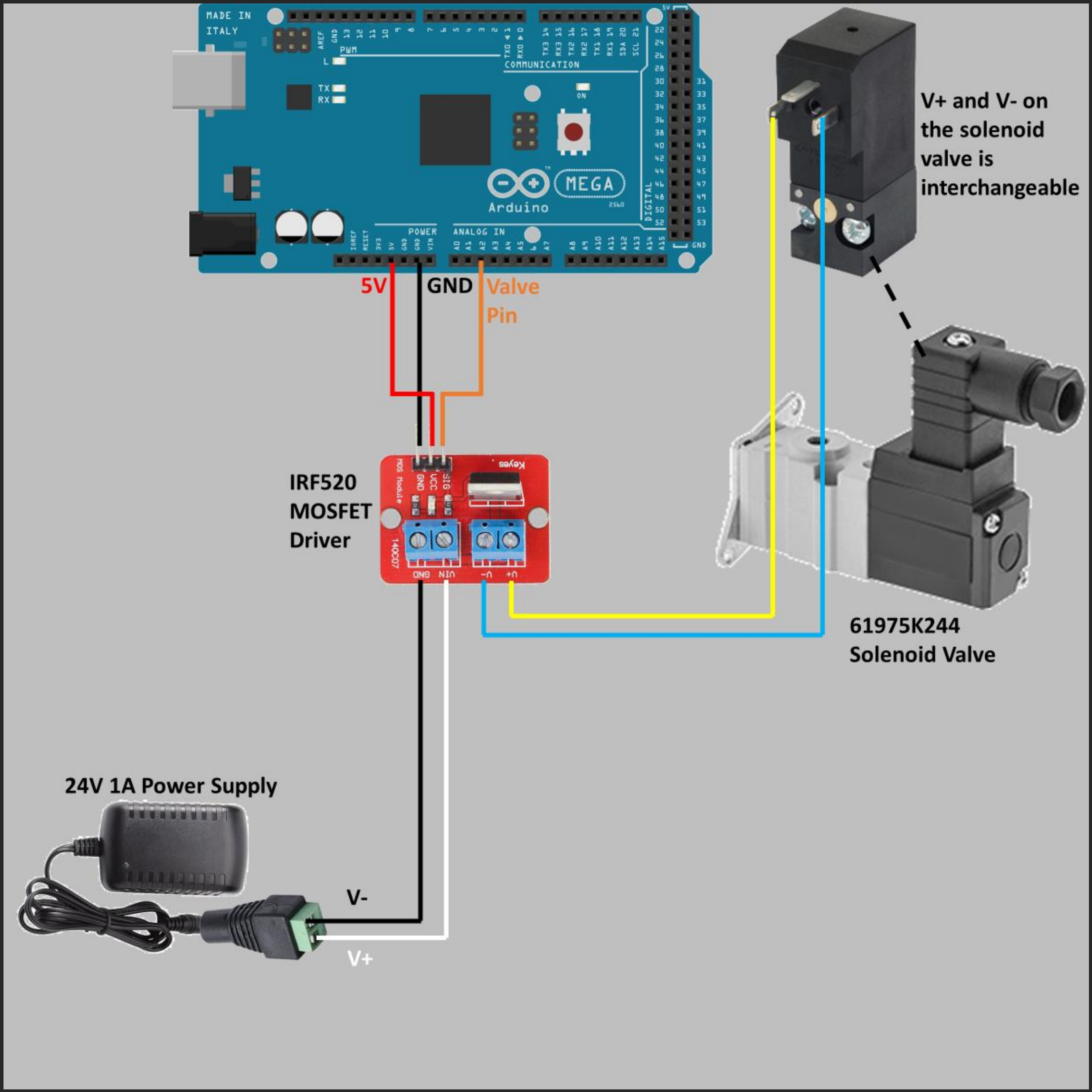
Wires from the Z actuator should run through the drag chains mounted on the X actuator, then through the drag chains mounted on the Y actuator, then down to the bottom surface where electronics are housed.

The step, direction, and limit pin connections to the microcontroller can be connected to any arbitrary GPIO pin. The ID for the GPIO pin will be physically marked on the microcontroller. Be sure to set the appropriate Pin ID values for each of the three pins under "XYZ Stage Settings" in ModiPrint's desktop program.

Pneumatic Extruder: On the parts list, scroll down to the "Pneumatic Extruder" section to see which parts are required. Open the "Pneumatic Extruder" Sketchup file for part arrangements. Things to look out for:

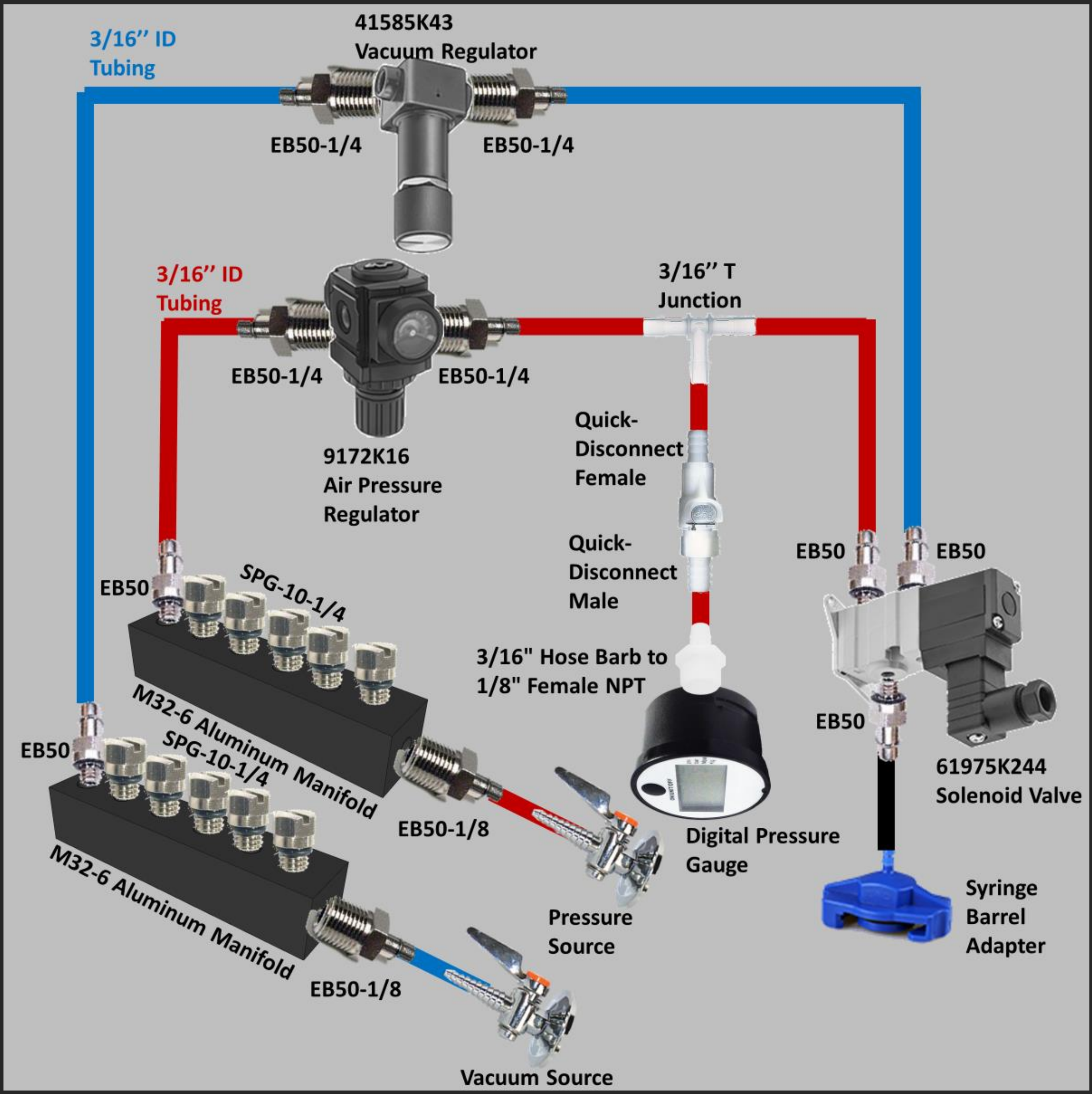
- Note that unlike the other sections, the printheads have various parts scattered across the printer. The solenoid valve is mounted on the top of the Z actuator and the syringe barrel mount at the bottom. The pressure regulator is mounted on the pneumatics panel behind the X actuator.
- The default syringe barrel mount is intended to hold a 3 mL syringe barrel. Typically, this material volume is enough for most prints. But if a 10 mL syringe barrel is needed, use the alternative 10 mL syringe barrel mount shown in the Sketchup file. If using 10 mL syringe barrels, edit the parts list by swapping 2x 3 Hole Joining Plates with 4x 2 Hole Joining Plates and add an additional 2x 10 mm M5 Screws and 2x M5 Tee Nuts. Be sure to purchase the 10 cc adapter assembly with filter trap instead of the 3 cc variant.

The wiring for the pneumatic extruder is as follows:



Wires from the pneumatic extruder should run through the drag chains mounted on the X actuator, then through the drag chains mounted on the Y actuator, then down to the bottom surface where electronics are housed.

The pneumatics for the pneumatic extruder is as follows:



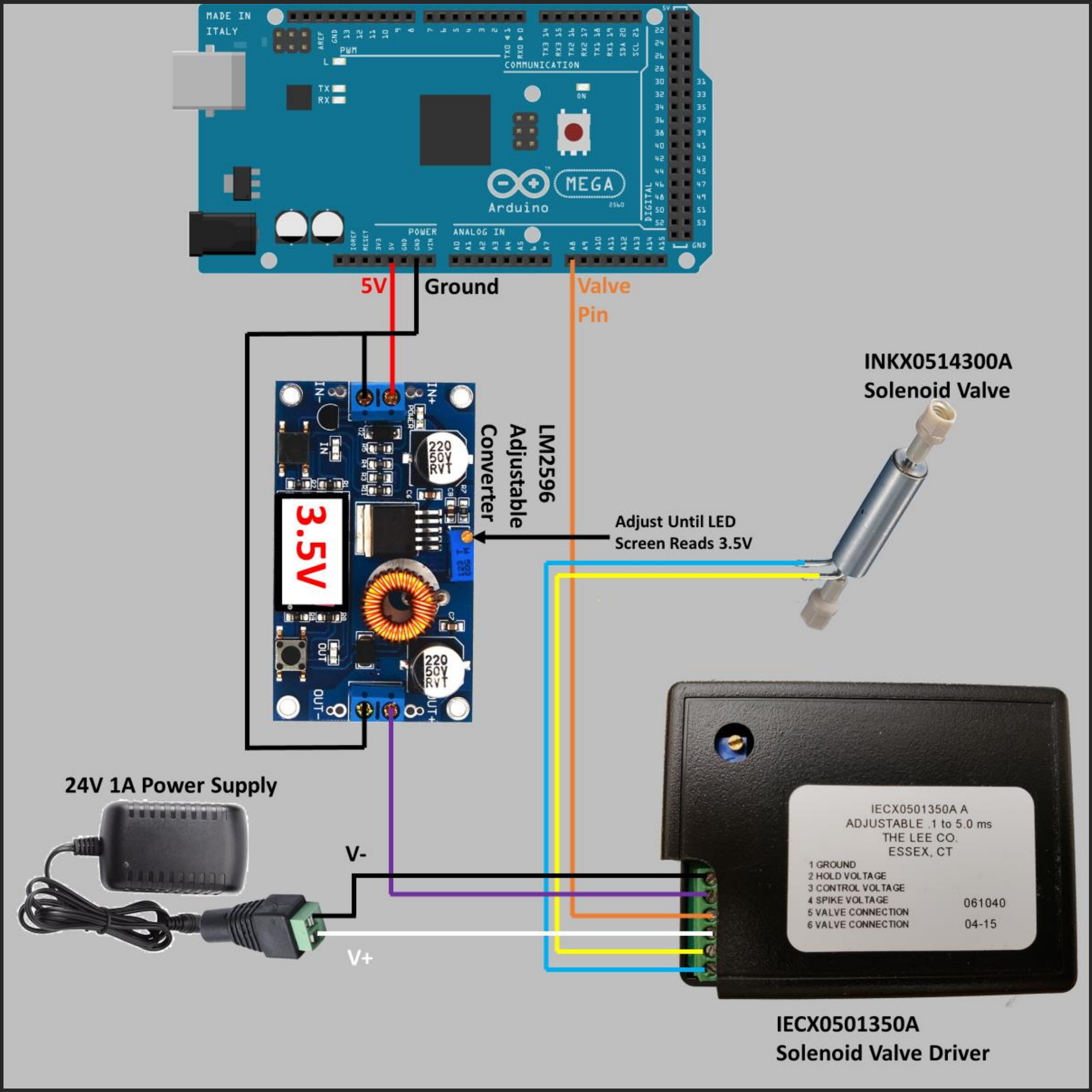
Multiple printheads of different types can share a single pressure and vacuum source via the M32-6 aluminum manifolds.

The digital pressure gauge is detachable and the quick-disconnect female fitting is airtight when disconnected. This allows a single pressure gauge to be used for multiple pneumatically driven printheads.

Microdispensing Inkjet: On the parts list, scroll down to the "Microdispensing Inkjet" section to see which parts are required. Open the "Microdispensing Inkjet" Sketchup file for part arrangements. Things to look out for:

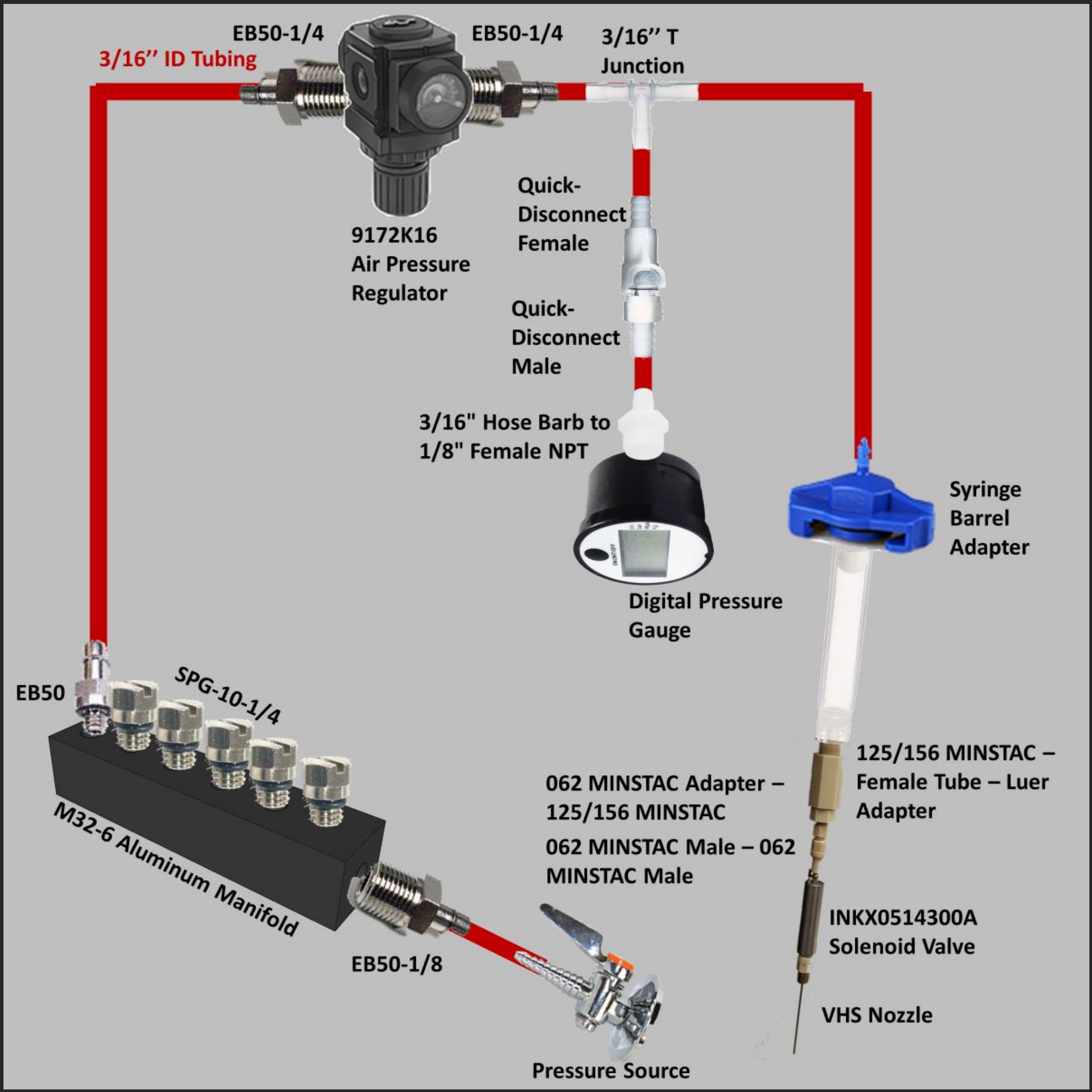
- Note that unlike the other sections, the printheads have different sections of their parts scattered across the printer. The syringe barrel mount is attached to the bottom of the Z actuator. The solenoid valve is attached to the syringe barrel via Luer lock. The pressure regulator is mounted on the pneumatics panel behind the X actuator.
- The default syringe barrel mount is intended to hold a 3 mL syringe barrel. Typically, this material volume is enough for most prints. But if a 10 mL syringe barrel is required, use the alternative 10 mL syringe barrel mount shown in the Sketchup file. If going this route, edit the parts list by swapping 2x 3 Hole Joining Plates with 4x 2 Hole Joining Plates and add an additional 2x 10 mm M5 Screws and 2x M5 Tee Nuts. Be sure to purchase the 10 cc adapter assembly with filter trap instead of the 3 cc variant.
- The solenoid valve on the microdispensing inkjet is extremely sensitive to damage. Exposure to excess voltage or even mildly elevated temperatures can damage the valve. Consult its datasheet if exposure to harsh conditions is expected.

The wiring for the microdispensing inkjet is as such:



Wires from the microdispensing inkjet should run through the drag chains mounted on the X actuator, then through the drag chains mounted on the Y actuator, then down to the bottom surface where electronics are housed.

The pneumatics for the microdispensing inkjet is as follows:



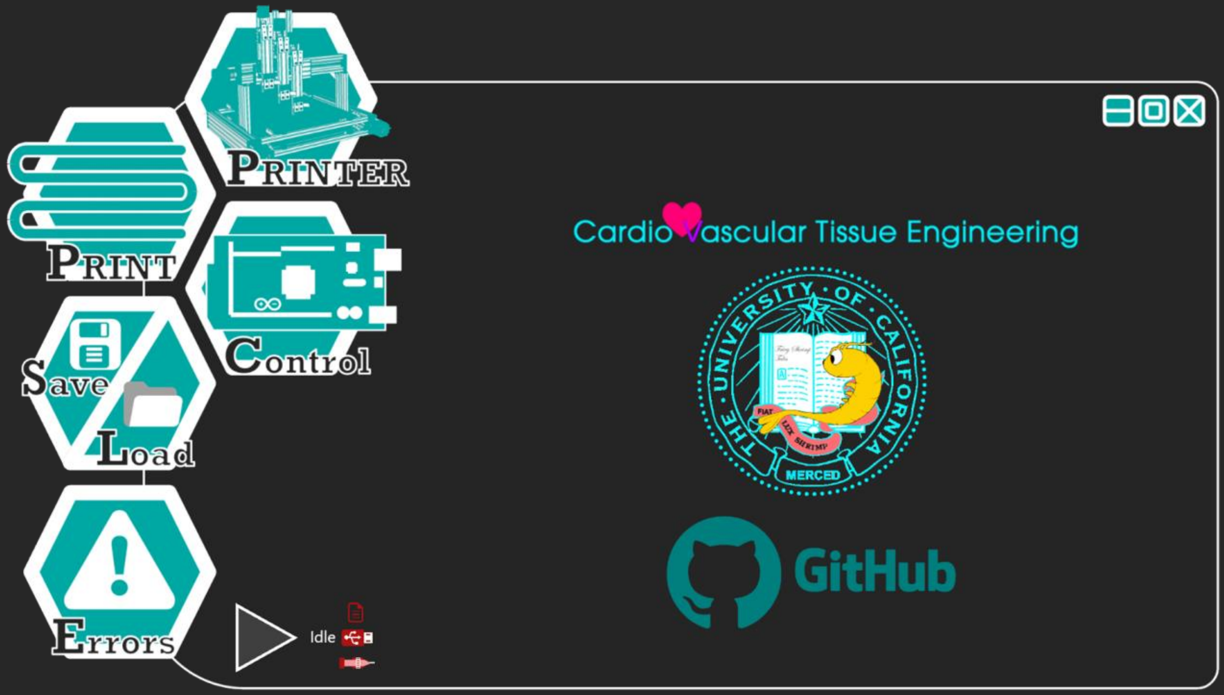
Multiple printheads of different types can share a single pressure source via the M32-6 aluminum manifolds.

The digital pressure gauge is detachable and the quick-disconnect female fitting is airtight when disconnected.

Software Installation

Desktop Program: Windows operating system is required to run the desktop program. If Windows is not the native operating system, then Windows can be emulated with various free software. Consult your institution's IT department for Windows emulation.

The desktop program is included with the documentation under the "ModiPrint Desktop" folder. To begin the program, simply run ModiPrint.exe. Do not move any files inside the "ModiPrint Desktop" folder.



Microcontroller Program: ModiPrint's firmware can be purchased on ModiPrint's website. The microcontroller comes pre-loaded with the program and should not be used outside the instructions of this guide. This program is the only part of the system that is not open source.

Software Operation

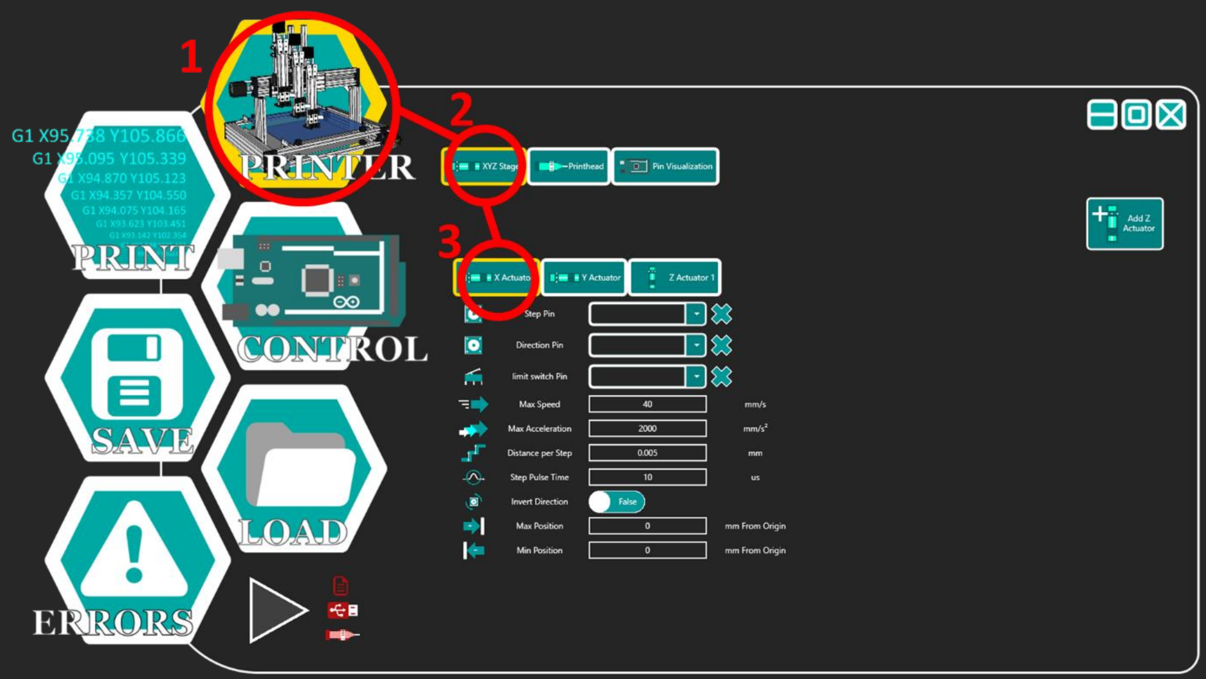
Printer Settings

The “Printer” menu on ModiPrint’s desktop program sets the hardware configuration of the printer. It allows the software to know how many actuators and printheads are on the printer as well their capabilities. Setting the values in this menu is the first action when first starting the program and only needs to be done once. After setting these parameters, be sure to save them.

Note that if following ModiPrint’s hardware guides, then the software’s default values for these actuators and printhead parameters should be acceptable. Changing these settings is only necessary when pushing the limits of the hardware or using modified hardware. Reading about how to set these parameters will only be necessary to push the capabilities of this machine or to support custom hardware. The exception is the Step, Direction, and Limit Pin parameters for the XYZ actuators and the Attached Z Actuator parameter for the Z actuators which are not defaulted to any value and must be set by the user.

XYZ Stage Parameters

Navigate to the “Printer” menu then to the “XYZ Stage” tab followed by the “X Actuator” tab to begin setting parameters for the X actuator.



Step Pin: Pin ID for the direction pin of this actuator. With default hardware, this actuator's DQ542MA stepper driver will have a wire connected to its "PUL-(PUL)" slot. That wire should be connected to the microcontroller via a GPIO pin that is physically marked with this pin ID.

Direction Pin: Pin ID for the direction pin of this actuator. With default hardware, this actuator's DQ542MA stepper driver will have a wire connected to its "DIR-(DIR)" slot. That wire should be connected to the microcontroller via a GPIO pin that physically marked with this pin ID.

Limit Switch Pin: Pin ID for the limit switch pin of this actuator. With ModiPrint hardware, this actuators' limit switches will have wires attached to the "NO" (normally open) slot. These wires should be connected to the microcontroller via a GPIO pin that is physically marked with this pin ID. Although not recommended, no selection in this parameter is allowed and the actuator will not stop when ranging out of bounds.

If the pin ID is not displayed in the dropdown, then that same pin ID is set somewhere else. The same GPIO pin cannot be set to two operations. Clicking the X to the right of the pin ID dropdown will clear the selection and allow use of that pin ID elsewhere.

Max Speed (mm/s): Maximum speed that this actuator can traverse in mm/s. With default hardware, the recommended setting for X and Y actuators is 40 mm/s. Z actuators have 15 mm/s.

Higher max speeds decrease operation time and enable higher print speeds for lower extrusion filament diameters. This value should be set as high as possible given the following criteria:

1. If the max speed exceeds the microcontroller's computational capacity, the "^Cyc" error message will appear. Depending on other workloads being processed, the microcontroller handles up to ~10,000 steps/s (50 mm/s at 1/8th microstepping).

2. Screw-based actuators are prone to violent vibrations at higher speeds which may affect precision. If the screw for the actuator is noticeably "bouncing" during operation, consider lowering the max speed value.

3. Once a limit switch is triggered, the printer will come to a completely stop without deceleration. This puts stress on the device and should be minimized if possible.

Max Acceleration (mm/s^2): Maximum acceleration that this actuator can traverse in mm/s^2. Higher accelerations decrease operation time and reduces cornering filament diameter during extrusion printing. High acceleration values are particularly important for maintaining consistent extrusion filament diameters during complex prints.

The default Z actuators are less stiff and torquey than the X and Y actuators. This necessitates that their max speed and max acceleration are lower. Most print operations do not occur in the Z direction. Prints should ideally be built with multiple XY layers as typically enforced by slicing software.

This value should be set as high as possible given the following criteria:

1. Higher acceleration values require higher torque. If this torque is not met, the motor will miss steps leading to inaccurate operation. To quote the GRBL wiki: "individually test each axis with slowly increasing

(acceleration) until the motor stalls." A motor stall will be obvious with loud, unpleasant sounds. Note that finer microstepping values decrease torque and necessitate lower acceleration values.

2. Higher acceleration values introduce higher stress and potential inaccuracies to the device. If there seems to be too much "kick" when the XYZ stage change directions, consider lowering this value.

With ModiPrint hardware, the recommended setting for X and Y actuators is 2000 mm/s^2. Z actuators have 25 mm/s. The hardware can handle higher acceleration at the user's discretion.

Distance per Step (mm): The distance traversed with each step of an actuator motor in mm. Also known as actuator resolution.

$$Distance\ per\ Step = \frac{Screw\ Pitch * Stepper\ Driver\ Microstepping}{Motor\ Steps\ per\ Revolution}$$

With default hardware, theoretical resolution at 1/8th microstepping is 5 µm for XY actuators and 2.5 µm for Z actuators. Note that actual resolution will differ very slightly from the theoretical.

Step Pulse Time (µs): To quote the GRBL wiki: "If the pulses are too long, you might run into trouble when running the system at very high feed and pulse rates, because the step pulses can begin to overlap each other." With default hardware, 10 µs should suffice. With custom modifications, consult the stepper motor datasheet for an optimal step pulse time.

Hovering over dropdowns and text boxes will cause a tooltip to pop up. The information in this section of the guide can be found within these tooltips in the software.

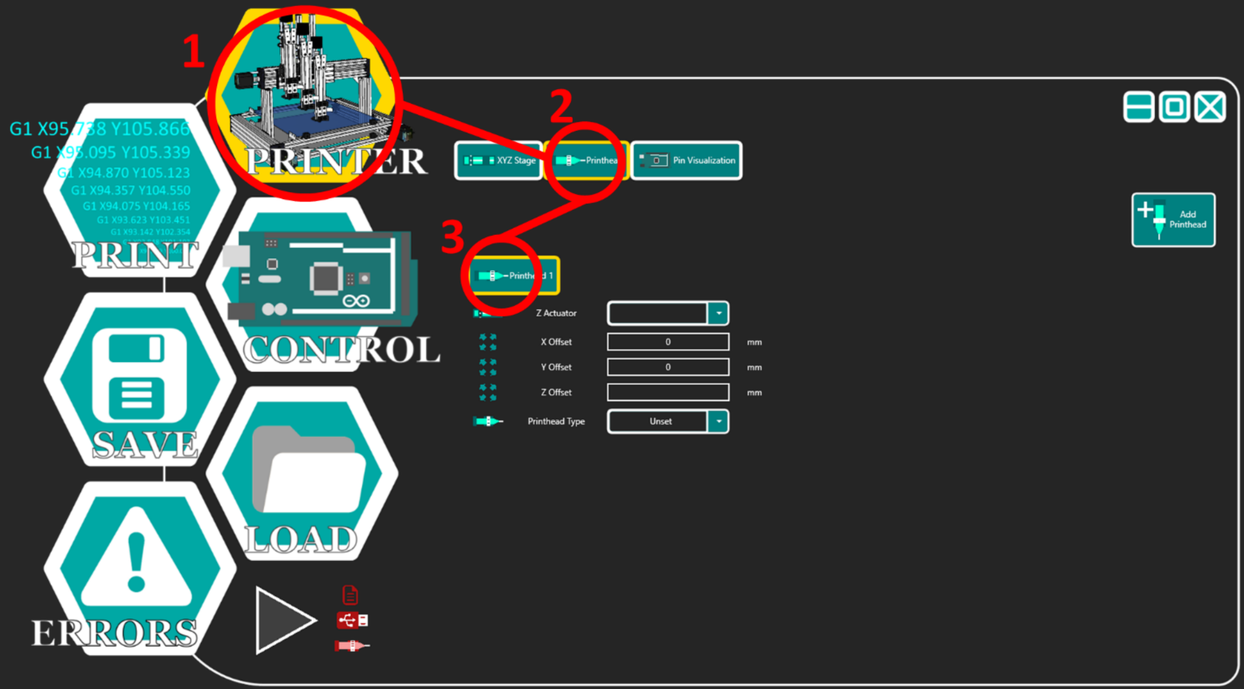
Invert Direction: Determines what direction is considered positive movement. Toggling this value will invert the direction of movement for this actuator with "True" setting a clockwise rotation as positive and "False" setting counterclockwise. Depending on the orientation of the actuators, the intended direction of positive movement may be mirrored (such as Z actuators moving down on positive movement values). Default values for all actuators are set for default hardware. Only radically different XYZ stage designs necessitate changing this setting.

Max Position (mm): For X and Y actuators, this represents the position value of a limit switch relative to an arbitrarily set origin in mm. For Z actuators, this represents the distance from the upper limit switch to the lowest print position. This parameter is intended to be automatically set during the calibration process. Only manually set these values if a more precise process than automatic calibration is being used.

Min Position (mm): For X and Y actuators, this represents the position value of a limit switch relative to some arbitrary origin in mm. For Z actuators, this represents the lowest print position and should always be zero (origin). This parameter is intended to be automatically set during the calibration process. Only manually set these values if a more precise process than automatic calibration is being used.

Printhead Parameters

Navigate to the “Printer” menu then to the “Printhead” tab followed by the “Printhead 1” tab to begin setting parameters for the first printhead.



Z Actuator: The Z actuator this printhead is physically attached to. All operations done by this printhead will use this Z actuator for vertical movement. Note that the Z actuator needs to be set in the “XYZ Stage” tab before it can be set here.

X, Y, and Z Offsets: The X, Y, and Z distance of this printhead from some arbitrary origin in mm. Offsets from all printheads must be set relative to the same origin. Specifically, the offsets of some printhead A subtracted by the offsets of some printhead B are the distances between printhead A to printhead B.

This parameter is intended to be automatically set during the calibration process. Only manually set these values if a more precise process than automatic calibration is being used.

Printhead Type: Determines if this is a valve or motor-driven printhead. With default hardware, the pneumatic extruder and microdispensing are considered valve printheads. Any printheads driven by a stepper motor are considered motorized.

Different printhead type settings will change which parameters that appear on the interface.

Valve Printhead – Valve Pin: Pin ID for the Valve Pin of this actuator. With default hardware, the printhead valve’s IRF520 MOS Driver will have a wire connected to its "SIG” slot. That wire should be connected to the microcontroller via a GPIO pin that is physically marked with this pin ID.

Motorized Printhead - Step Pin: Pin ID for the step pin of this actuator. The stepper driver should have a wire connected to its signal input. That wire should be connected to the microcontroller via a GPIO pin that is physically marked with this pin ID.

Motorized Printhead - Direction Pin: Pin ID for the direction pin of this actuator. The stepper driver should have a wire connected to its direction input. That wire should be connected to the Arduino MEGA microcontroller via a GPIO pin that is physically marked with this pin ID.

Motorized Printhead - Limit Switch Pin: Pin ID for the limit switch of this actuator. The limit switch should have a wire connected to normally open input. That wire should be connected to the microcontroller via a GPIO pin that is physically marked with this pin ID. Although not recommended, no selection in this parameter is allowed and the actuator will not stop when ranging out of bounds.

Motorized Printhead - Max Speed (mm/s): Maximum speed that this actuator can traverse in mm/s. Higher max speeds decrease operation time. This value should be set as high as possible given the following criteria:

During extrusion, motorized printhead speeds and accelerations are matched to the movement of the XYZ stage such that all motors start and stop simultaneously while extruding a line segment. The motorized printhead's traverse distance will be much smaller than the XYZ stage's traverse distance for motorized syringes or thermoplastic extruders. Therefore, the motorized printhead's speed and acceleration values typically operate well below appropriate speed settings.

1. If the max speed exceeds the microcontroller's computational capacity, the "^Cyc" error message will appear. Depending on other workloads being processed, the microcontroller upper limit is just over 10,000 steps/s.

2. Screw-based actuators are prone to violent vibrations at higher speeds which may affect precision. If the acme screw is noticeably "bouncing" during operation, consider lowering the max speed value.

3. Once a limit switch is triggered, the printer will come to a completely 100 to 0 stop without deceleration. This puts stress on the device and should be minimized if possible.

Motorized Printhead - Max Acceleration (mm/s^2): Maximum acceleration that this actuator can traverse in mm/s^2. Higher accelerations decrease operation time.

This value should be set as high as possible given the following criteria:

1. Higher acceleration values require higher torque. If this torque is not met, the motor will miss steps leading to inaccurate operation. To quote the GRBL wiki: "individually test each axis with slowly increasing (acceleration) until the motor stalls." A motor stall will be obvious with loud, unpleasant sounds. Note that finer microstepping values decrease torque and necessitate lower acceleration values.

2. Higher acceleration values introduce higher stress and potential inaccuracies to the device. If there seems to be too much "kick" when the XYZ stage change directions, consider lowering this value.

Motorized printhead - Distance per Step (mm): The distance traversed with each step of an actuator motor. Also known as actuator resolution.

$$\text{Distance per Step} = \frac{\text{Screw Pitch} * \text{Stepper Driver Microstepping}}{\text{Motor Steps per Revolution}}$$

Note that actual resolution will differ very slightly from the theoretical.

Motorized Printhead - Step Pulse Time (µs): To quote the GRBL wiki: "If the pulses are too long, you might run into trouble when running the system at very high feed and pulse rates, because the step pulses can begin to overlap each other." Consult the stepper motor datasheet for an optimal step pulse time.

Motorized Printhead - Invert Direction: Determines what direction is considered positive movement. Toggling this value will invert the direction of movement for this actuator. This value should be set such that positive relative positions in the g-code causes the printhead to dispense and negative values cause retraction.

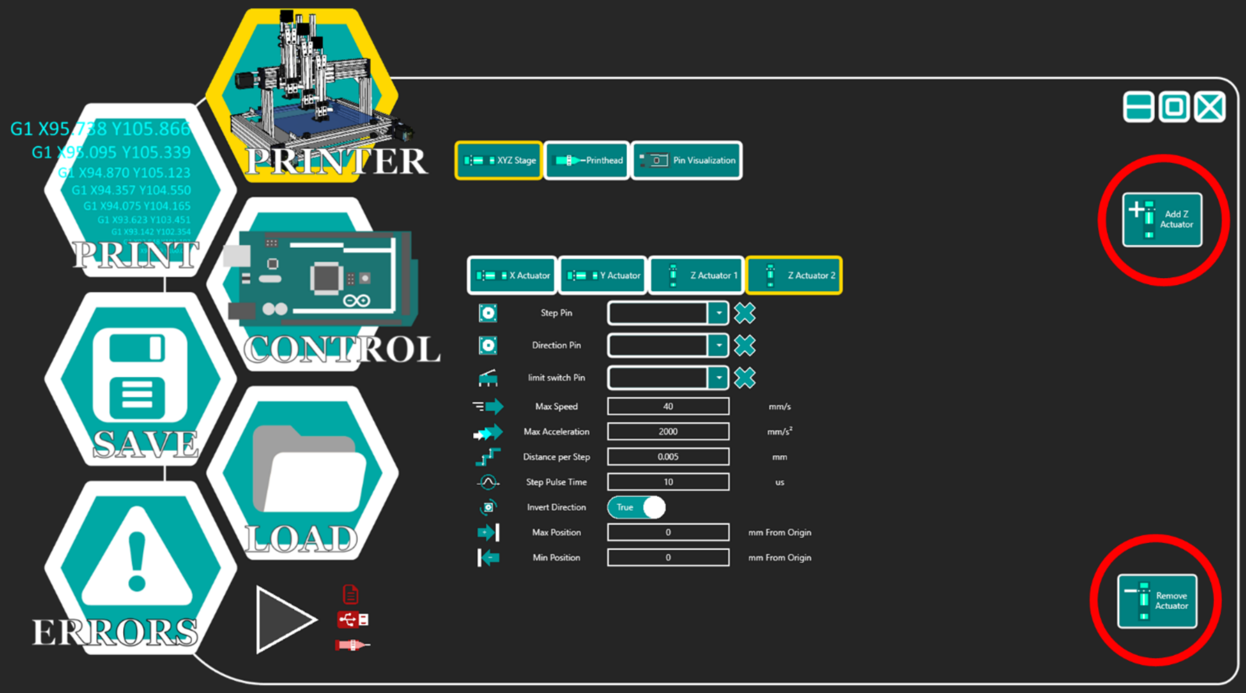
Max Position (mm): Traverse from the upper limit switch to the lowest print position in mm. This is intended to be automatically set during the calibration process. Only manually set these values if a more precise process than automatic calibration is being used.

Min Position (mm): Should always be zero (origin) and represents the lowest print position in mm. This is intended to be automatically set during the calibration process. Only manually set these values if a more precise process than automatic calibration is being used.

Multiple Z Actuators and Printheads

If the printer has multiple independent Z actuators and printheads, then the software must be set to recognize these actuators. Parameters will need to be set for each individual Z actuator and printhead.

For every additional Z actuator beyond the first, click on the “Add Z Actuator” button. To remove a specific actuator, navigate to the tab corresponding to that actuator then the “Remove Actuator” button. Adding and removing additional printheads involves buttons located in the same positions when the “Printheads” tab is selected. Note that deleted actuators and printheads will not remember its settings. All actuators contain their own individual parameters and all actuators set these parameters in the same fashion.


PRINTER
PRINT
CONTROL
SAVE
LOAD
ERRORS
XYZ Stage
Printhead
Pin Visualization
Add Z Actuator
X Actuator
Y Actuator
Z Actuator 1
Z Actuator 2
Step Pin
Direction Pin
limit switch Pin
Max Speed
40
mm/s
Max Acceleration
2000
Distance per Step
0.005
mm
Step Pulse Time
10
us
Invert Direction
True
Max Position
0
mm From Origin
Min Position
0
mm From Origin
Remove Actuator

Control and Calibration

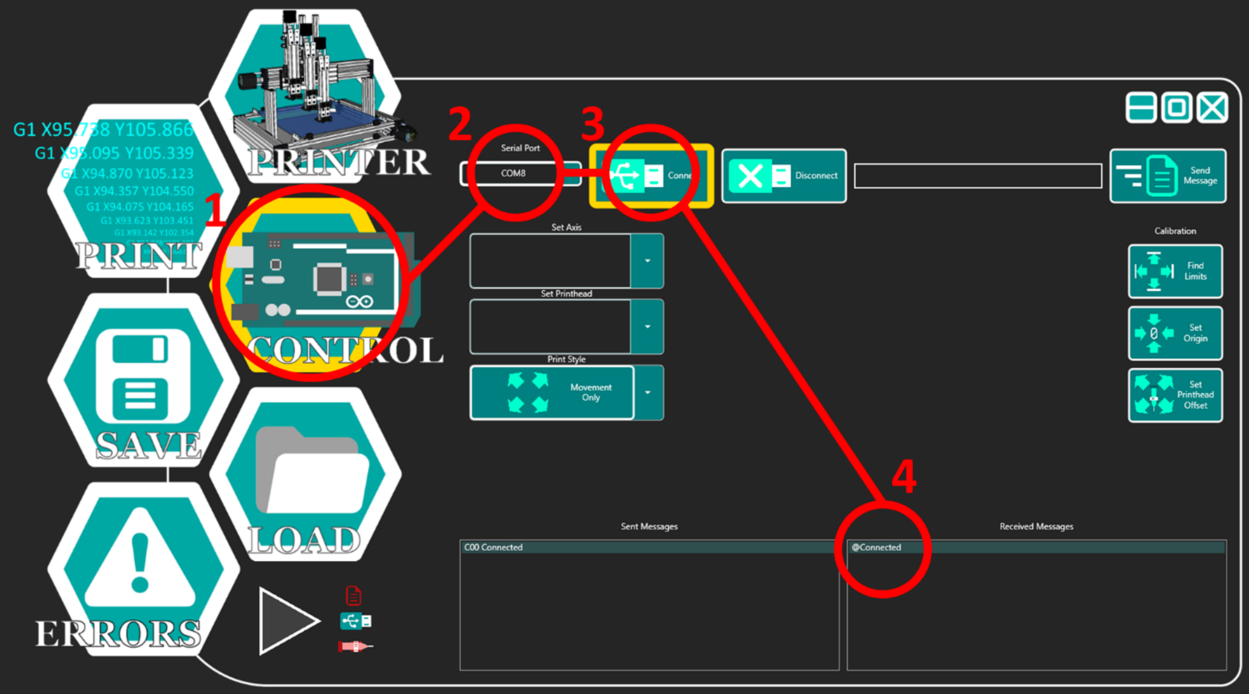
All features for communication with the microcontroller, manually operating the printer, or calibrating the printer can be found under the “Control” menu.

Serial Communication

ModiPrint’s desktop program communicates with the microcontroller via serial communication. Specifically, the desktop program streams lines of commands to the microcontroller. Whenever commands need to be streamed, ModiPrint queues that command to a list of outgoing messages. All commands streamed from executing a print sequence will be placed at the end of this queue and executed after other commands have been processed first. Non-calibration commands sent from the “Control” menu will be placed at the front of the queue and executed immediately.

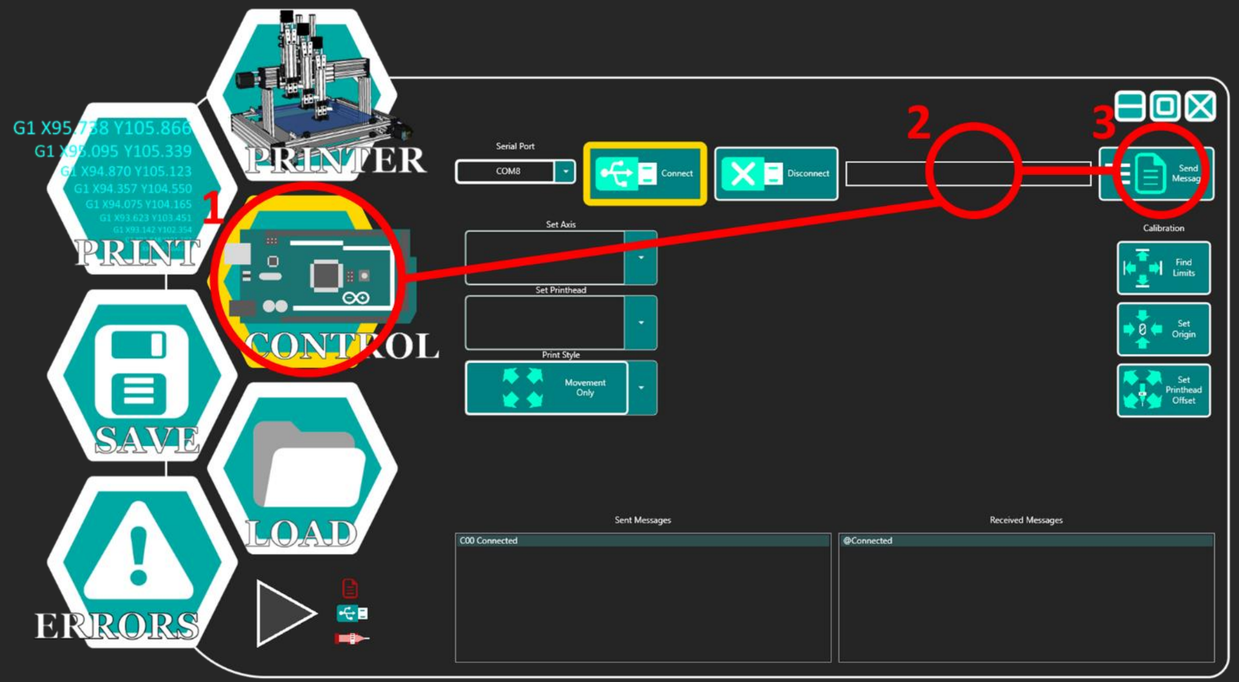
Connecting to the Serial Port: Connection to the microcontroller is necessary before any commands can be sent to and executed by the microcontroller. To connect:

1. Ensure that an Arduino MEGA, loaded with ModiPrint’s microcontroller software, is connected to the computer via a USB cable.
2. Wait 10 seconds for the microcontroller to boot up before attempting a connection.
3. Click on the “Serial Port” dropdown. Then select the serial port associated with the microcontroller.
    a. If the microcontroller is powered on and properly connected to the computer, then the correct port name should appear in the dropdown.
    b. The easiest way to identify which port is associated with the microcontroller, is to physically connect/disconnect the microcontroller and check for differences in the dropdown.
4. Once the correct port is selected, hit the “Connect” button.
    a. If successfully connected, the “Connected” button should be highlighted yellow, other elements of the user interface should appear, and “@Connected” should appear in the received messages list. If either of these things do not happen, it is recommended to restart the microcontroller (cut power or hit its reset button), restart ModiPrint’s desktop program, and repeat these steps.



Manually Serial Communication: Commands may be sent to the microcontroller directly by typing the command in the textbox to the left of the "Send Message" button then pressing that button. All supported commands are documented in the ModiPrint Commands Documentation section. Incorrect commands will return errors.

Note that manual communication should rarely be relevant as there are other, easier ways to send most commands (detailed in Manual Control).



Serial Messages Display: All messages sent to the microcontroller will be displayed under the "Sent Messages" list. All messages returning from the microcontroller will be displayed under the "Received Messages" list. All supported messages are documented in the ModiPrint Commands Documentation section.

Reading the serial message display is typically not necessary for normal operation. It's mainly there to show that communication is occurring and for debugging purposes.

Manual Control

Before continuing with manual control, set all printer parameters and established serial communication.

Manual control is a two-step process. First, specify operating parameters for the XYZ stage and printheads with the "Set Actuator" and "Set Printhead" dropdowns. Then UI elements will appear which will allow the operation of the equipment that was set prior. Only one actuator of each of the X, Y, and Z axes and only one printhead may be set at a time. Setting these equipment again will overwrite the equipment in use.

> Any time a new menu pops up, navigate back to the main control menu with located at the bottom left.

Set Actuator: Click on the "Set Axis" dropdown to set the actuator of an XYZ stage. A new window will appear for the specification of the speed and acceleration values which will be used for all proceeding commands. After specifying these parameters, click "Execute" or press the "Enter" key to set the actuator.

As each actuator is set successfully, the interface will create arrow-shaped buttons for directing movement of these actuators. Clicking on these arrow-shaped buttons will open a new menu for specification of the movement distance in the direction of the arrow. Movement commands with multiple actuators (diagonal arrows) will cause these actuators to start and end movement simultaneously. After specifying movement distance, click "Execute" to begin movement.

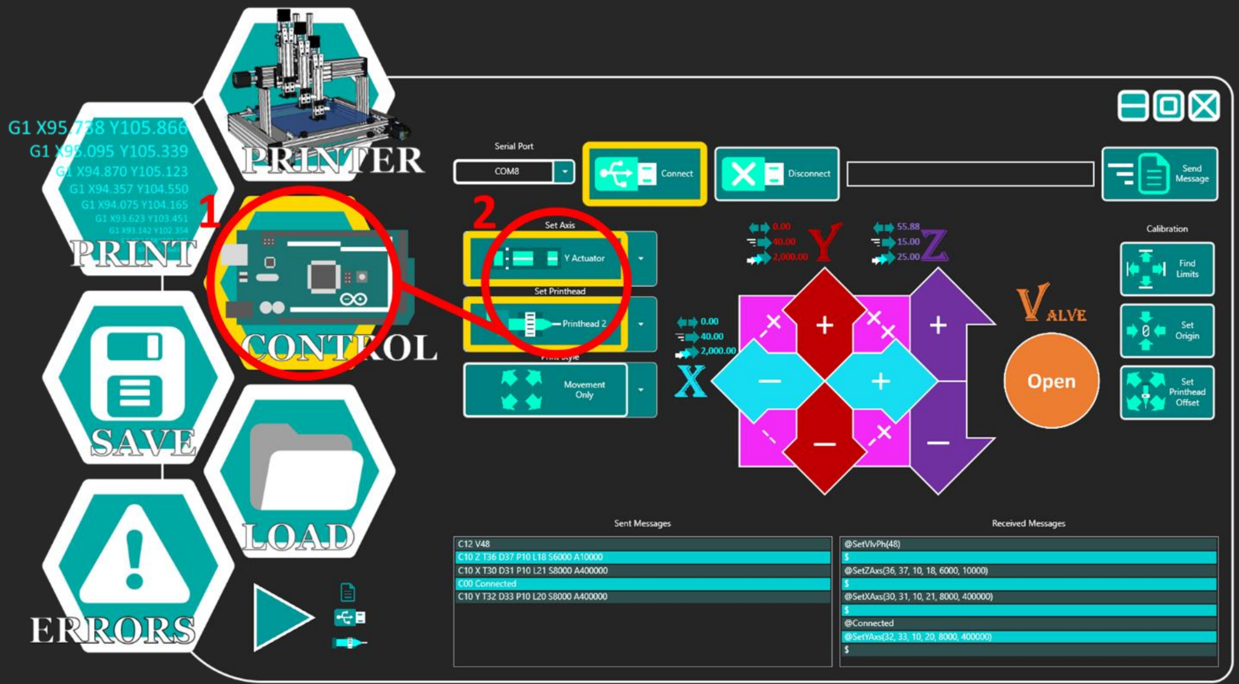
Whether these commands cause only movement without printing or movement with printing depends on what is set in the "Print Style" dropdown (see below).

> When equipment has been set and is being used for proceeding commands, they are highlighted yellow in the "Set Axis" and "Set Printhead" dropdowns.

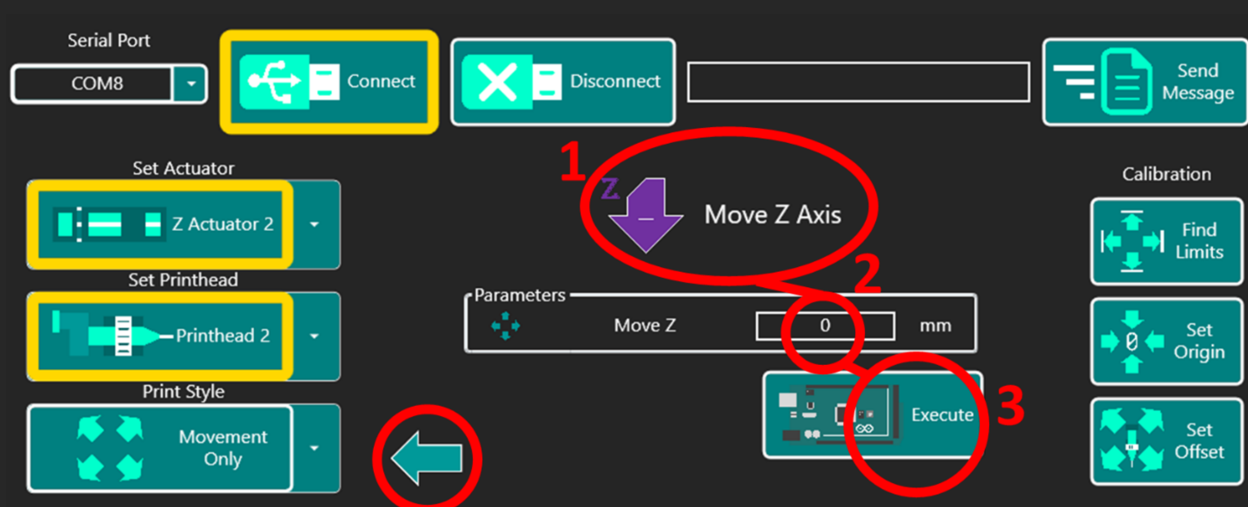
Set Printhead: Click on the "Set Printhead" dropdown to set the printhead and its associated Z actuator. Valve and motor-driven printheads will have different icons. A new window will appear for the specification of the printhead's parameters. For motor-driven printheads, set the speed and acceleration of the printhead motor. Valve printheads have no parameters to set. After specifying these parameters, click "Execute" or press the "Enter" key to set the printhead.

As each printhead is set successfully, the interface will create new buttons for operating these printheads. For motor-driven printheads, arrow buttons will appear that allow movement of the printhead motor just manually operating motors of the XYZ stage. For valve printheads, specify the amount of time that the valve will open. Or, if the valve open time is set to zero, the valve will remain open until told to close. When the valve is open, the circular valve control button will be highlighted light blue. When the button is highlighted, pressing it will close the valve (no menu will appear, the action will occur immediately).

The image below displays what the interface looks like if the X, Y, and Z actuators and a valve printhead is set.



The image below displays the submenu that appears when any action (such as XYZ stage movement, or printhead operation). This menu displays the action, any parameters for the action, and the "Execute" button that is pressed for the action to complete. It also contains a back button that exits the submenu.



Print Style: This dropdown affects whether movement (from the arrow-shaped buttons) will be accompanied by printing. These commands will execute movement without printing if the "Print Style" dropdown is set to “Movement Only”. The printhead will continuously dispense as the XYZ stage moves if the dropdown is set to “Continuous Print”. The XYZ stage will stop at specific intervals and dispense droplets at those intervals if the dropdown is set to “Droplet Print”. To see more about how droplet print works, see the Print Settings section. Note that any print actions will only occur if a printhead has been set prior.

Status Displays: As each actuator and printhead is set, there will be status displays next to their respective UI buttons. Next to the XYZ stage actuators and motor-driven printhead:

Displays the position of the actuator relative to the origin.

Displays the max speed value currently used by the actuator.

Displays the acceleration currently used by the actuator.

Calibration

Before continuing with calibration, set all printer parameters and established serial communication. Calibration features also require that the actuators have limit switches installed. It is recommended that calibration begin with the “Home” button, before calibrating printhead offsets with “Set Offsets”. Do not perform any other actions in between the calibration process.

Home: This feature will find the minimum and maximum position values of the XYZ stage actuators. Then it will center the XYZ stage.

Begin the calibration process by clicking on the “Home” button to the right and a new menu will appear. This menu allows setting of maximum speeds (in mm/s) for each actuator during this process. Since the actuators will physically hit the limit switches, these speeds should be set low. With default hardware, the recommended settings are 10 mm/s for each actuator.

This menu also allows setting the origin and centering the XYZ stage. The origin location will be determined by the menu’s X and Y positions where (X0, Y0) is at the physical center of the XYZ stage.

Hit “Execute” or press the “Enter” key and the following sequence will occur:

1. Retract all Z actuators until the upper limit switches are hit. Z actuator min and max position parameters will not be recorded.

2. Move X actuator to both upper and lower limit switches. Record min and max position parameters.

3. Move Y actuator to both upper and lower limit switches. Record min and max position parameters.

4. Move to the center of the XYZ stage.

Set Offset: This feature sets the min/max positions of the Z actuators and offsets of each printhead.

1. Install nozzles on each printhead as if printing is about to occur.

2. Set a printhead from the "Set Printhead" dropdown.

3. Move the tips of each nozzle precisely to a specific position in 3D space (for instructions, see the manual control section).

4. Hit the "Set Offset" button.

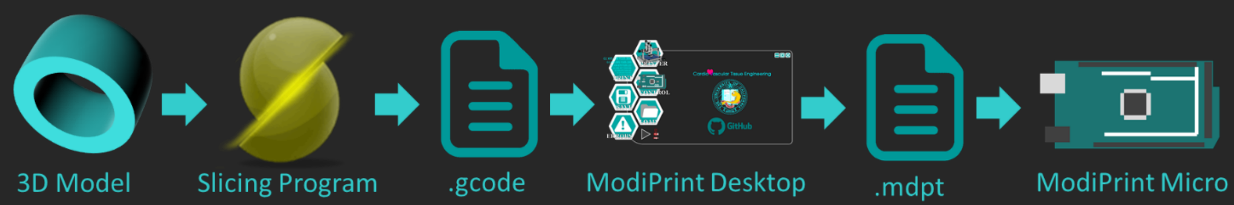
5. Repeat steps 3-5 for all printheads, maneuvering them to the exact same position.

With multiple printheads, all offsets must be set sequentially with no other actions taken in between. It is recommended to use the Save/Load feature after calibration to keep these offset values. Although these offset values should be accurate for multiple print sessions, periodic calibration is recommended.

Generating G-code from CAD

ModiPrint Workflow

ModiPrint's microcontroller requires a specialized set of commands found in no other system. Therefore, in order to integrate computer-aided designs (CAD) into the workflow, CAD files must be converted to tool paths via other slicing software in the form of G-code. ModiPrint's desktop program is compatible specifically with RepRap's flavor of g-code and converts this language into a specialized one used only by ModiPrint. This allows ModiPrint to be compatible with and leverage the functionality of a variety of CAD and slicing software. ModiPrint commands are finally streamed to the microcontroller during operation.



Generating g-code from CAD files is recommended if the geometry is too complex to map by hand. If performing simple operations, or operations that aren't printing sliceable shapes, then see the section on generating g-code by hand.

CAD Models

Any prints with complex shapes can first be drawn with CAD software. These CAD files will need to be converted to .gcode files before use in ModiPrint's desktop program. There are numerous CAD software out there and it recommended that users select and learn one with the features for their specific design. Since the various CAD software operate quite differently, there will not be a specific example in this guide.

Some universally applicable advice:

- Drawings need to be solid, 3D objects, and not just the walls of a hollow shape.
- Different CAD and slicing software support different file formats. Certain file formats also provide more detailed information than others.
- ModiPrint uses mm as the standard unit, therefore g-code files must be generated with the mm unit.

Note that generating tool paths for 3D printing is finnicky. Some combinations of CAD and slicing software generate better tool paths than others. The online 3D printing community is also a great online resource for such recommendations.

Alternatively, 3D models can be found online. The NIH 3D Print Exchange is an excellent resource for biology related 3D models and models of CT scans. There are also numerous sources like Free3D for models of any kind.

Slicing Software

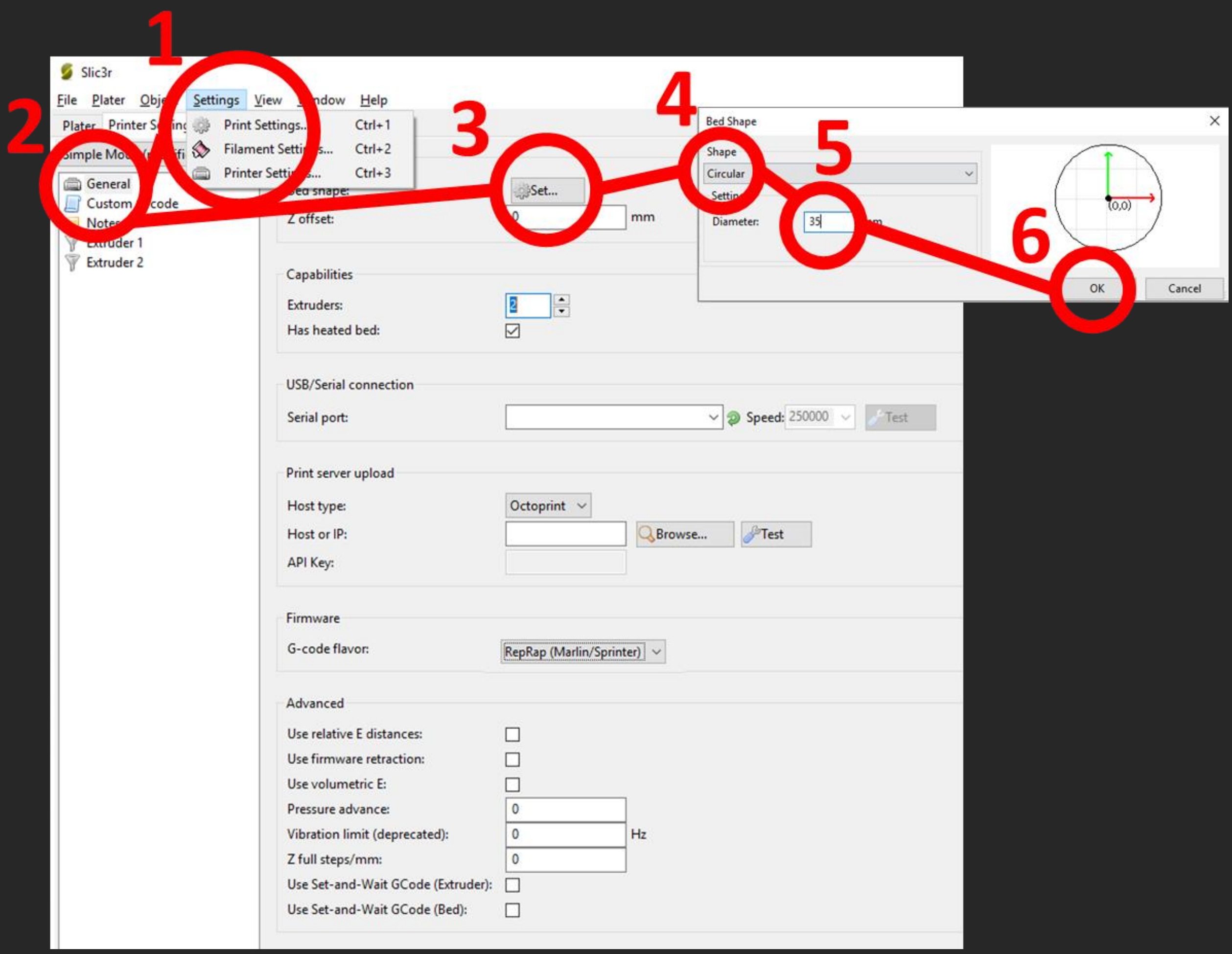
Slicing software is necessary to convert CAD files into tool paths. While there are many options out there, this guide recommends Slic3r. This section of the guide will example how to operate Slic3r in the context of ModiPrint. Note that other slicing programs have similar settings and this guide is mostly translatable to other programs. This guide will be using Slic3r 1.3.0 which can be downloaded here.

ModiPrint only cares about the tool path (movement path of the printhead) generated from slicing software. Therefore, many features on slicing software will not be relevant to ModiPrint. For all g-code relevant to ModiPrint, see here.

The following sections of this guide will example how to generate settings for a 2-material print of solid concentric cylinders.

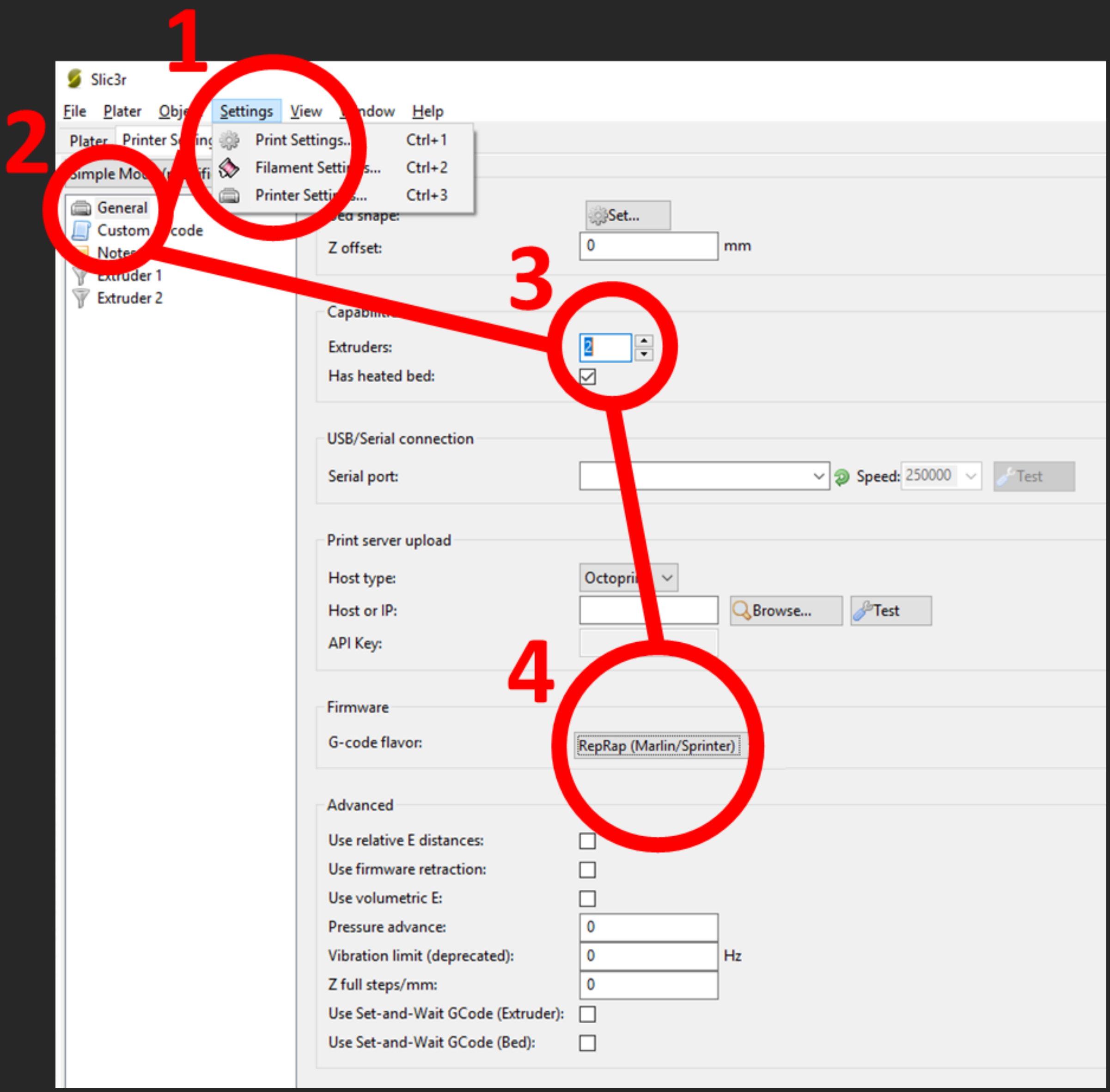
Printer Bed: Open the “Settings” menu, then select “Printer Settings”, then “General”. Clock the “Set” button for bed shape. The example in this guide will be printing into a 35 mm petri dish, so the shape should be set to circular and the diameter to 35 mm. Then hit “Ok”.

The center of circular beds is set as the origin. ModiPrint will begin the print at the origin, so the printhead’s physical start location (the center of the petri dish) is set here. In the case that a rectangular bed is set, the origin would default to the bottom left of the bed. In that hypothetical case, ModiPrint would begin the print at a very awkward bottom left spot that’s likely outside the petri dish (the print will fail).



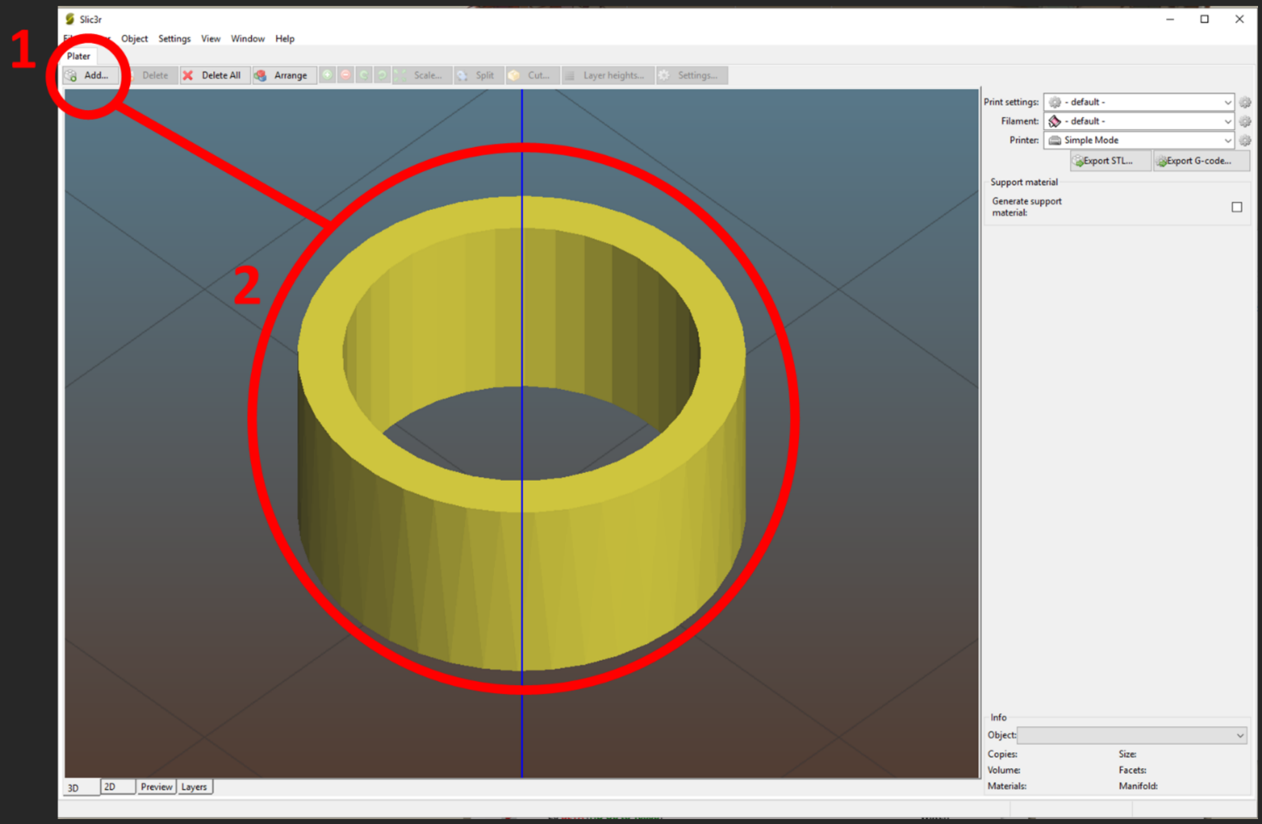
CAD Files and Multiple Materials: Open the “Settings” menu and select “Printer Settings”. Go to the “General” tab and set the number of extruders equivalent to the number of materials to be used. In this example, the value is set to two. This means that two different materials will be printed.

Go to the “Firmware” box and set the g-code flavor to RepRap. ModiPrint’s desktop program only reads this flavor of g-code.

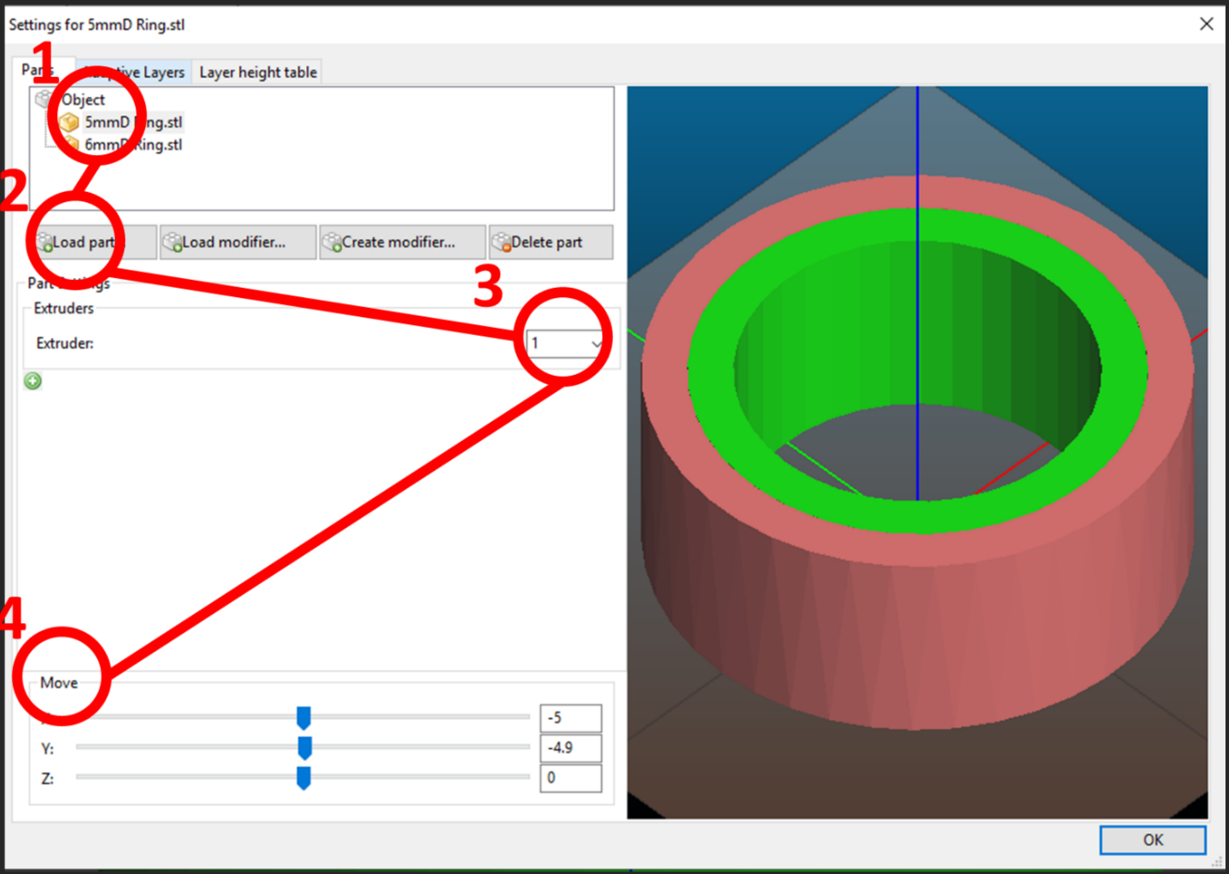


Click the “Plater” tab then the “Add” button on the top left of Slic3r interface. For simplicity, this guide will example a .stl file of a 5 mm outer diameter, 4 mm inner diameter, 5 mm height cylinder, though any shape is applicable. If uploaded successfully, an image of the 3D model should appear. Double left-click on the 3D model to bring up a settings menu.

Note that Slic3r supports a variety of file formats each with various advantages and disadvantages.



When printing multiple materials, a different CAD file will be required for each material. Upload other materials by selecting the name of the first CAD file under “Objects”. Then select “Load Part” then the appropriate file. Assign each part to an extruder under “Extruders”. If the parts are not placed correctly, their position can be adjusted with the sliders under the “Move” box.



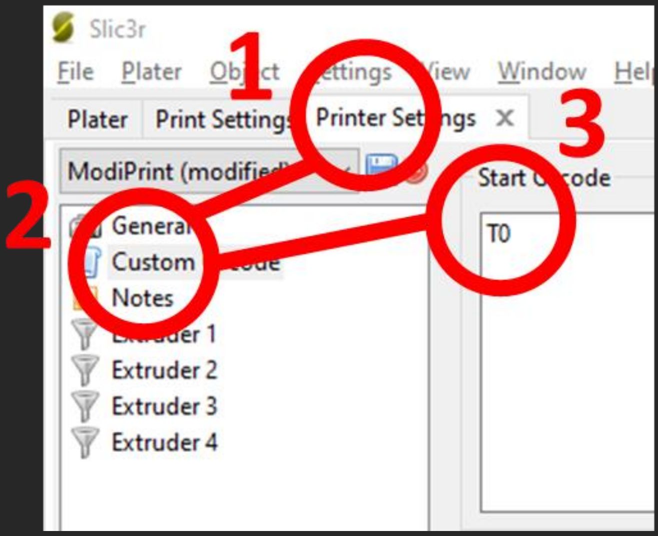
For this example, a 6 mm outer diameter, 5 mm inner diameter, 5 mm height cylinder has been uploaded. The inner cylinder is set to extruder 1 and the outer cylinder is extruder 2. Since both parts' positions were already concentric in AutoCAD, the sliders in the "Move" box does not need to be adjusted. Note that Slic3r will begin the print at the origin position, and that the printed parts need to be centered at the origin.

What is a material referring to exactly? Each material refers to a different set of tool paths that will be executed with different print settings. Each material will process their own tool paths with any combination of different speeds, different printheads, different print styles, etc. Most of these parameters will be set within ModiPrint's desktop program "Material Settings".

For this specific example with concentric cylinders, the inner cylinder is set to extruder 1 and the outer cylinder to extruder 2. In the g-code output, the command "T0" will appear before any commands for printing the outer cylinder. Likewise, "T1" will appear before any commands for printing the inner cylinder. When reading this g-code file, ModiPrint's desktop program will switch to different sets of print parameters on T0 and T1.

Start G-code: Open the "Settings" menu and select "Printer Settings". Click on the "Custom G-code" tab and under the "Start G-code" textbox, the default value should be "T0." If the first CAD object to be printed (dimensions are closest to the origin) has its extruder set to Extruder 1, then leave the "Start g-code" textbox with the default value of "T0". Otherwise, delete "T0" and any other contents from the "Start G-code" textbox.

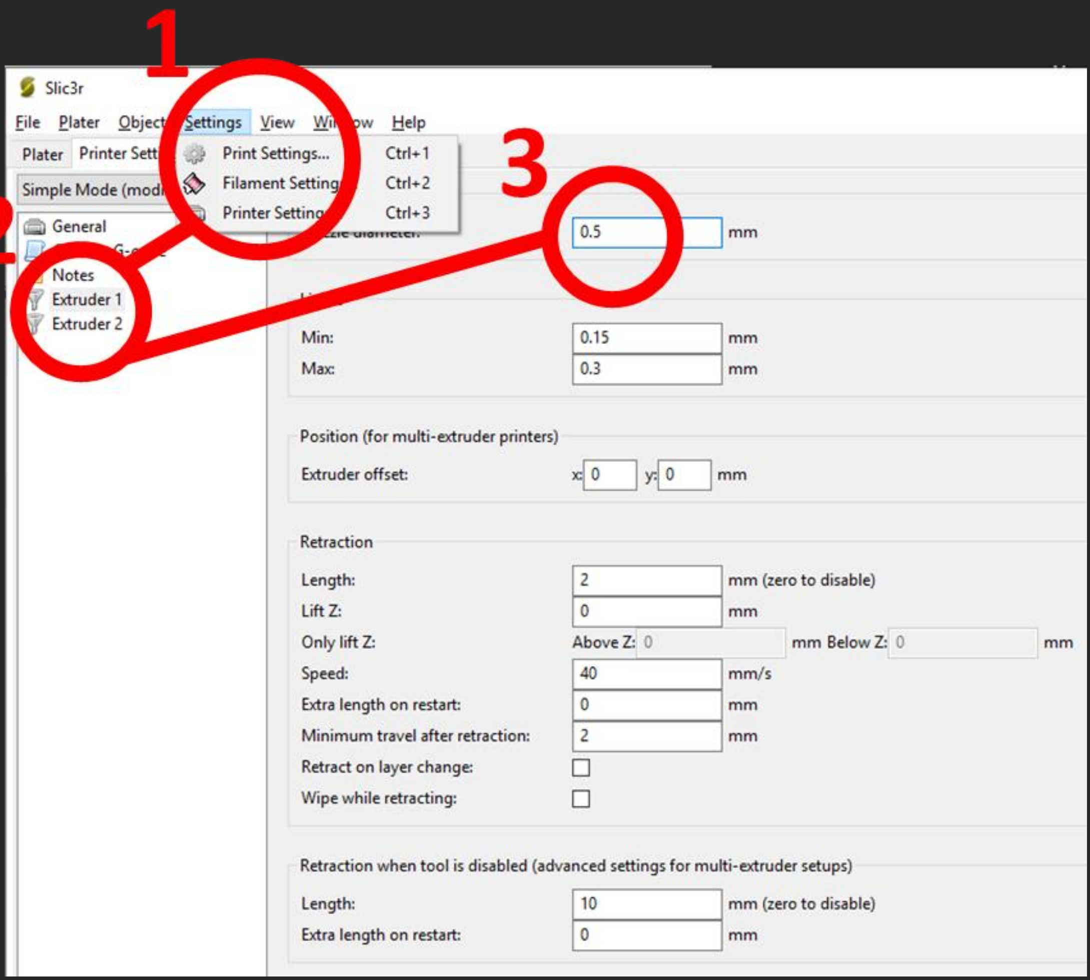
In this concentric cylinder example, the start g-code should be set to “T0” because the innermost cylinder will be printed first.



This step is necessary because the “Start G-code” parameter adds lines to the beginning of the g-code file that Slic3r will generate. In this case, the “T0” line will be interpreted by ModiPrint as the command to switch to a Printhead with the T0 g-code ID.

Strangely enough, if the first object to be printed uses Extruder 1 (corresponding to T0), Slic3r will not automatically insert “T0” into the beginning of the g-code file. Therefore, if “T0” is not in the start g-code textbox, no printhead will be set and ModiPrint will erroneously ignore all g-code until a “T#” command appears. If the first object to be printed uses any other extruder than Extruder 1, Slic3r will automatically insert “T#” into the beginning of the G-code file. Slic3r will always inserts the start g-code sequence to the beginning, therefore, a “T0” start g-code in this instance will cause an unnecessary printhead switch at the beginning.

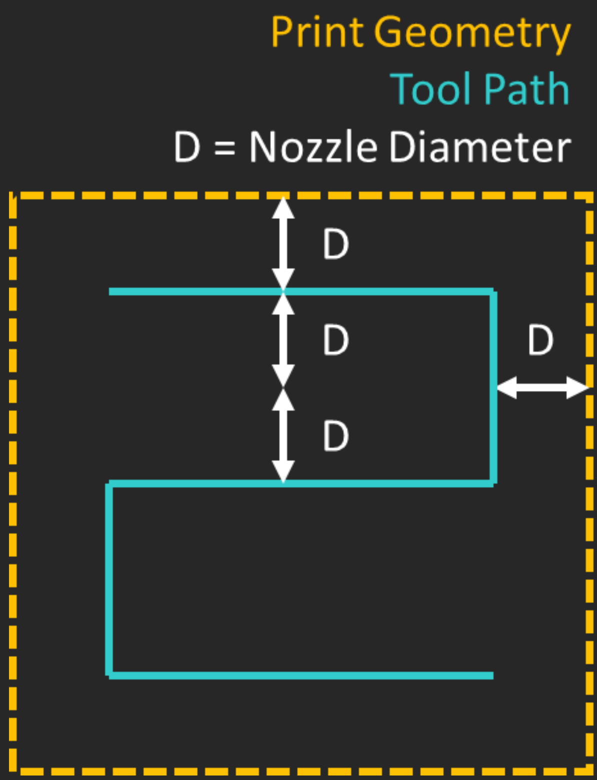
XY Tool Path Spacing: Open the “Settings” menu and select “Printer Settings”. Go to the tabs named “Extruder #”. Each extruder corresponds with a different material in the ModiPrint desktop program. For each extruder, set the nozzle diameter corresponding to the XY tool path spacing of each material. For this example, extruder 1 is set to 0.1 mm and extruder 2 to 0.5 mm.



What does tool path spacing mean exactly? Slic3r corresponds the nozzle diameter to the size of extruded filaments. For extruded filaments to not overlap, Silc3r varies the spacing of the tool path. Therefore, this nozzle diameter parameter is used to set the spacing of tool paths.

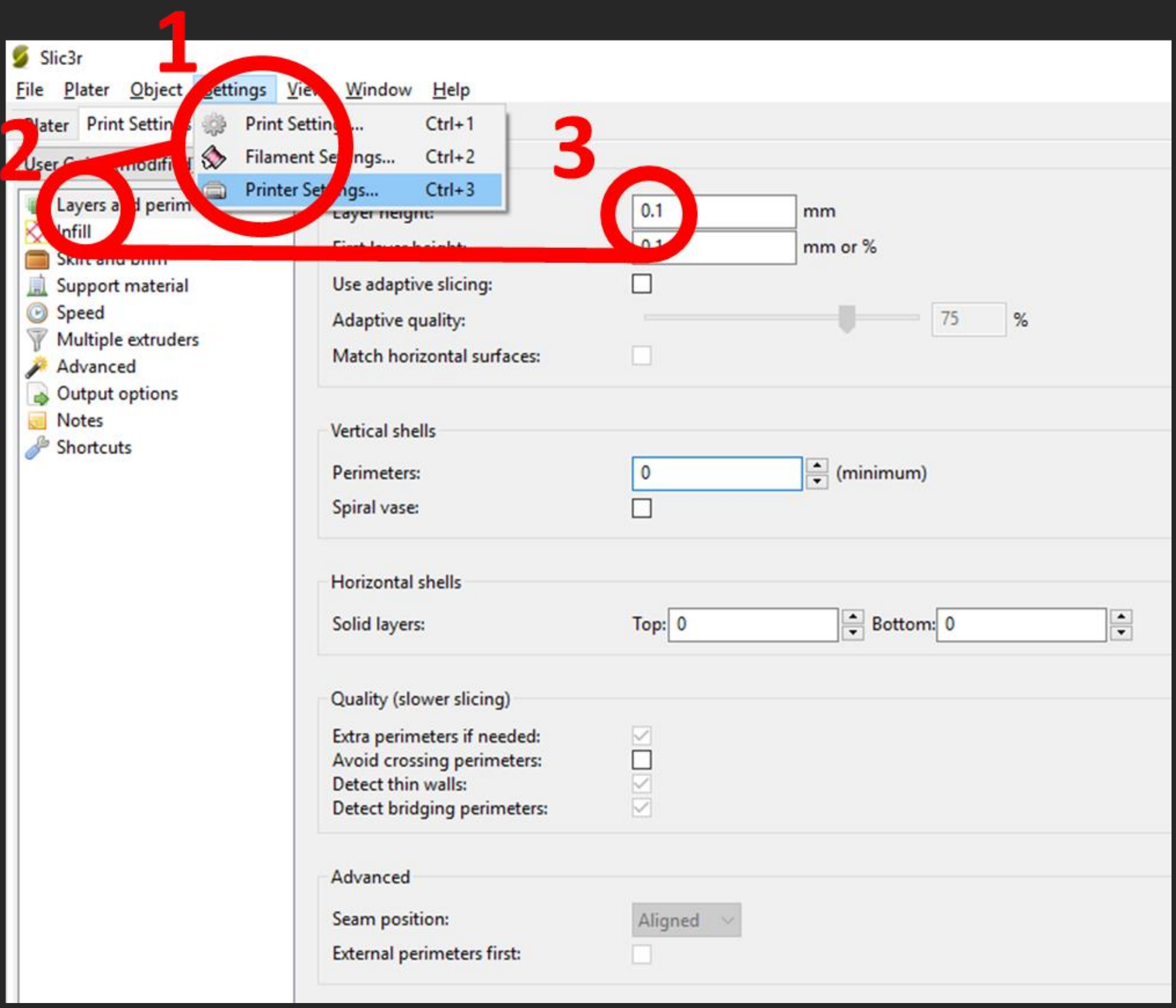
For example, a print of a simple 3x3 mm square with a single Z-layer. Using a 90 degree rectilinear fill pattern and 100% infill (no gaps in the solid structure) the tool path will look like this:

Do not confuse what is being said here with the "Filament Diameter" parameter in Slic3r. This refers to the diameter of unextruded filaments and is used to calculated extrusion rates which is irrelevant in the context of ModiPrint.



A real example would not be this simple, and various settings can alter the tool path (especially near the boundaries). Also note that nozzle diameter only affects XY spacing and not Z spacing. To see the results of the nozzle diameter setting on the XY spacing of the cylinder example, skip ahead to the section detailing how to view tool paths.

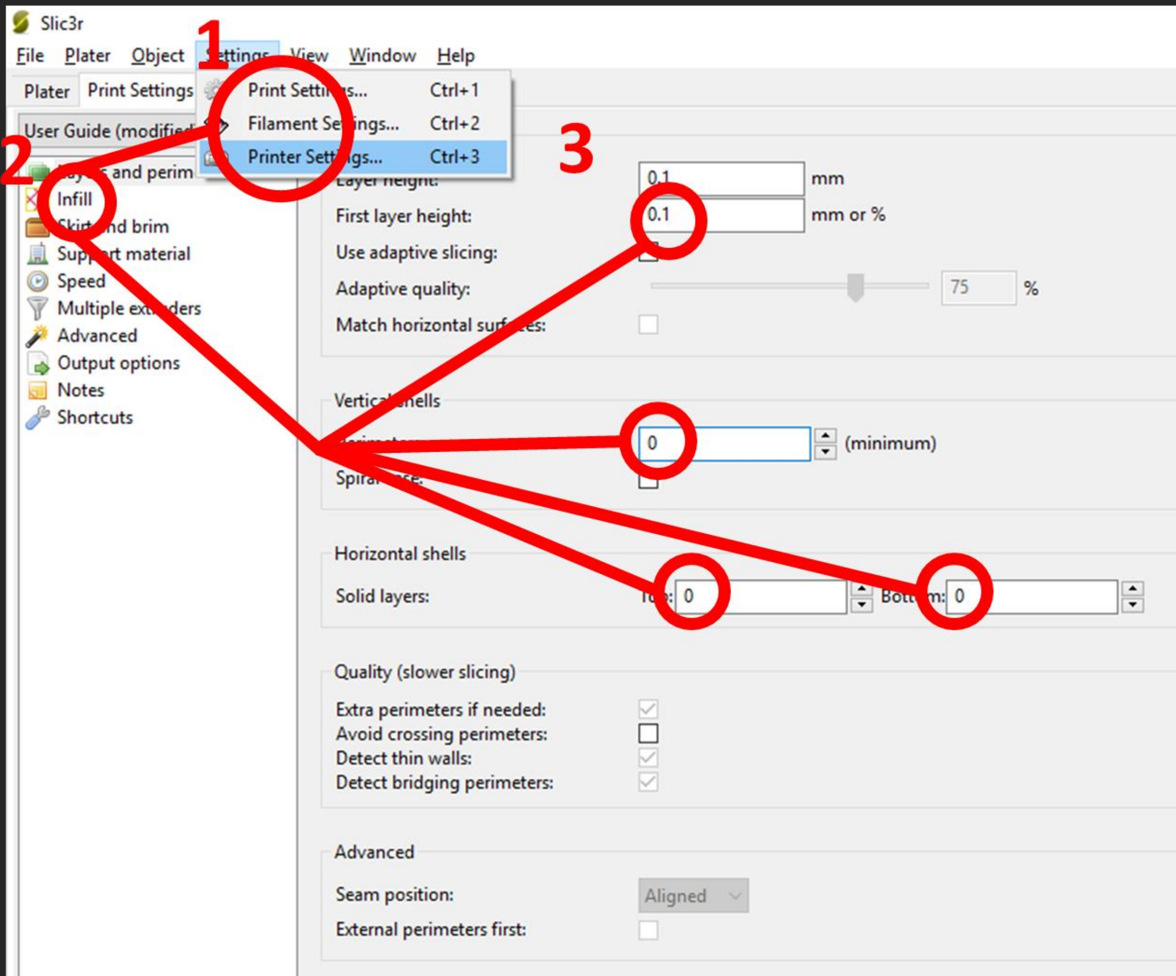
Z Tool Path Spacing: To set the Z tool path spacing, open the "Settings" menu and select "Print Settings" tab. Select "Layer and perimeters". The "Layer Height" setting corresponds to the Z tool path spacing. This setting is universal to all extruders/materials.



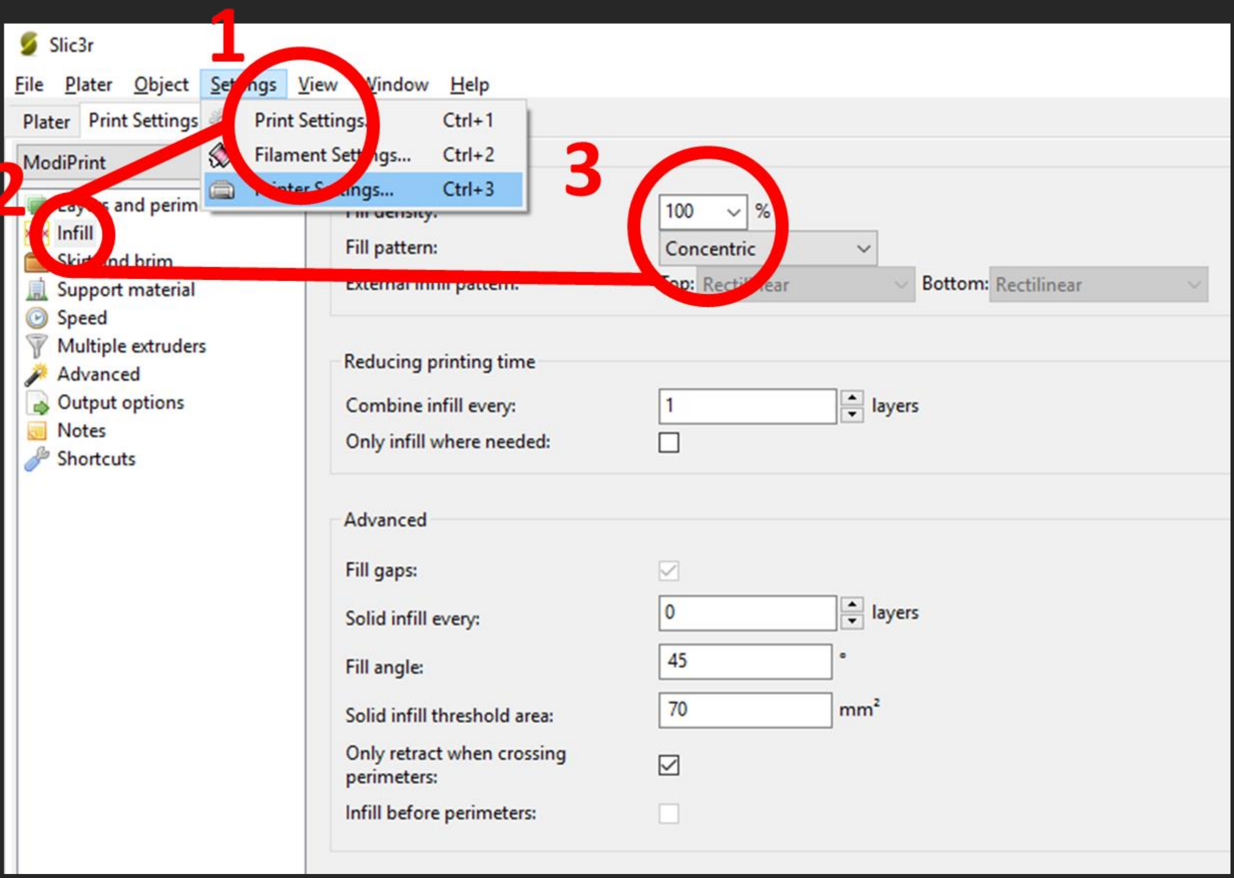
Note is that this example's 0.5 mm nozzle setting with a 0.1 mm layer height is a ridiculous combination of settings. The filament would have an extremely wide but short cross section to fit such parameters. An unrealistic setting only used here to demonstrate the effects of nozzle diameter on the resulting tool path.

Perimeters: Slicing software typically differentiates between print settings for exterior layers (perimeters) of the object and interior layers (infill). These features are important for conventional 3D printing as the exterior of thermoplastic prints need to be solid and the interior can be hollow. However, bioprinting typically would not use perimeters as most tissue are not solid exteriors with hollow supports.

For the concentric cylinder example uniform, solid, concentric rings will be printed. Therefore, there would be no need for perimeters, only infill. To disable perimeters, go to the "Settings" menu, then select "Print Settings", then "Layers and perimeters." Under the "Vertical Shells" and "Horizontal Shells" boxes, set all perimeter and layer values to zero. This will disable exterior layers. Since our concentric rings will be uniform, the "First layer height" setting should be the same as the "layer height" setting (0.1 mm).



Infill: The infill print settings will determine how the majority of the print is generated. Go to the “Settings” menu, then select “Print Settings”, then “Infill”. “Fill pattern” will determine the pattern of the tool path while “Fill density” determines the percent volume by which the structure will be filled with print material. “Fill density” is another way to adjust tool path spacing with lower percentage values resulting in larger distances between filaments. For visual representations of the different “Fill pattern” settings, see the Slic3r manual.



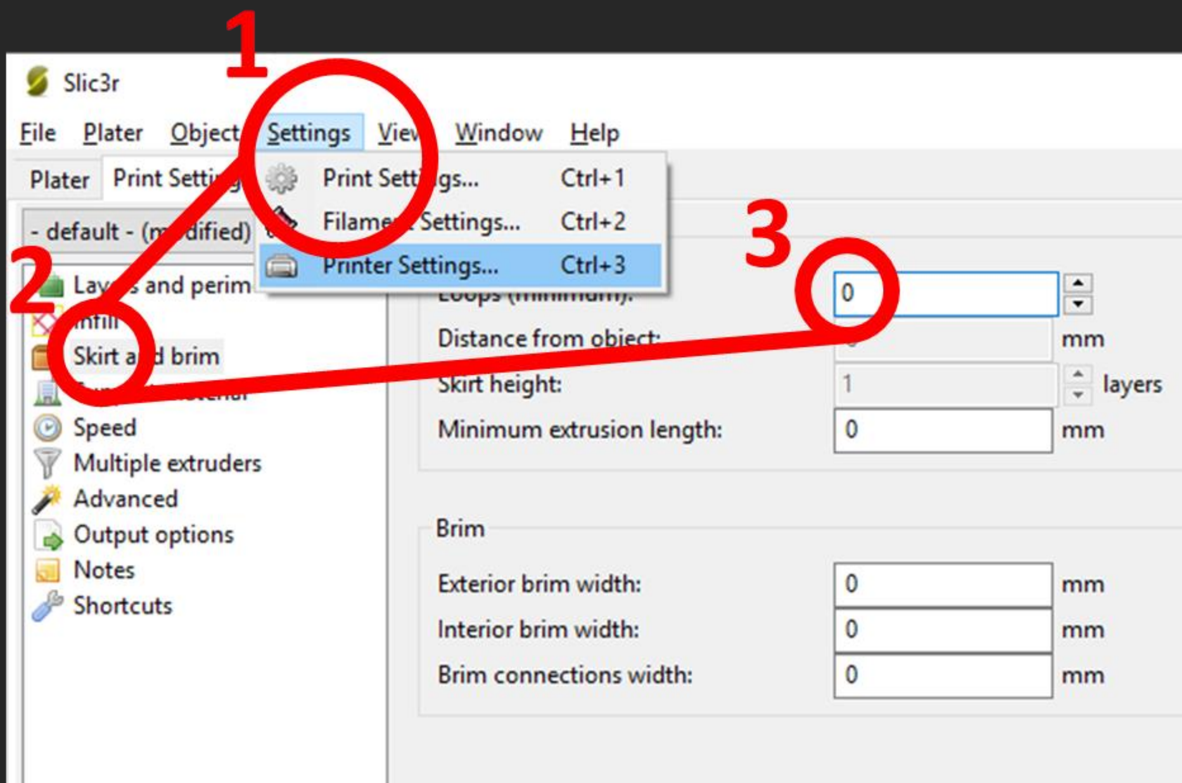
The example in this guide aims to generate solid, concentric cylinders, Therefore, 100% infill will generate a solid structure. A concentric fill pattern conforms to the geometry of a cylinder and will ensure little tool path overlap.

Another consideration is that cornering speeds are an important consideration in infill pattern selection. For pneumatic extrusion printheads, tool paths with tighter corners (like rectilinear infill pattern) will result in more inconsistent movement speeds and consequently more inconsistent filament diameters. A concentric fill pattern for a circular geometry will result in milder corner angles but an increased frequency of corners.

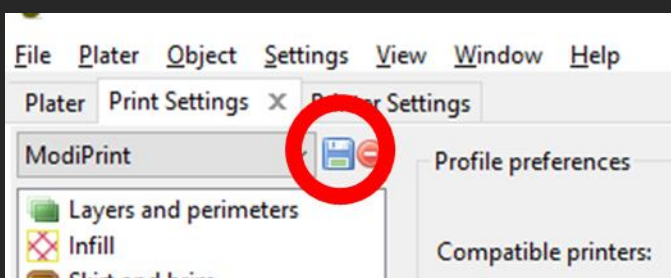
Skirt and Brim: A skirt is an outline of the 3D printed part that does not touch the printed part itself. It is printed at the lowest level (print surface) before the part itself to help prime the extruder and expose potential issues. Brims also outline the 3D printed part but physically touches the part at the lowest level. It can be used to support the printed part or, like the skirt, debug issues.

Typically, these settings are not necessary for bioprinting. But if the material/printhead has similar issues to thermoplastic extrusion (adhesion, warping at the lower level and edges, requires priming), then consider using these features as well as the features under “Settings” ➔ “Printer Settings” ➔ “Support material”.

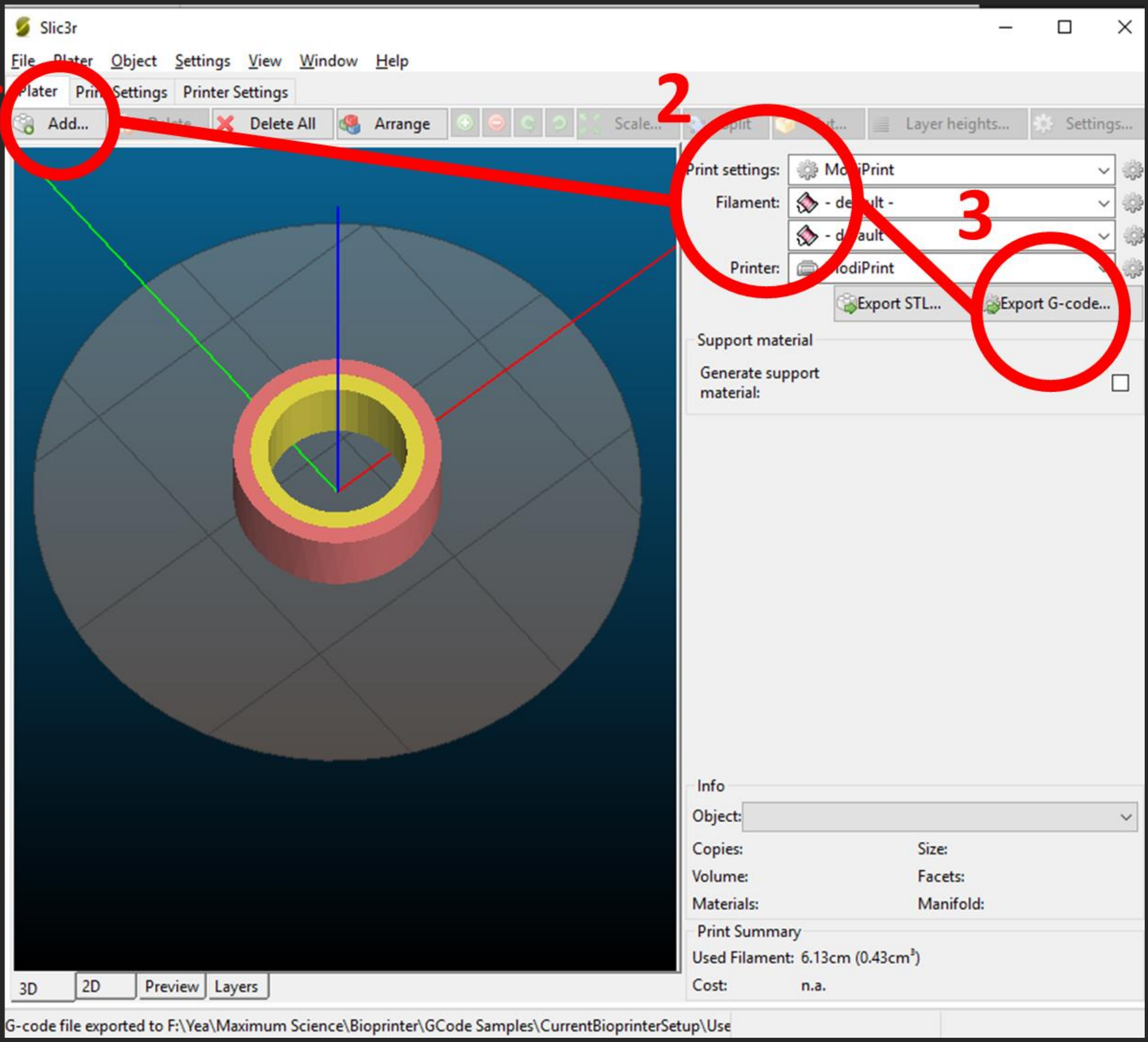
For the example in this guide, these features will not be used. Go to the “Settings” menu, then select “Printer Settings”, then “Skirt and Brim”. Brim is by default disabled, but the “Loops” parameter under the “Skirt” box must be set to zero to disable skirts.



Saving Slic3r Settings: Under both the "Print Settings" and "Printer Settings" tabs (accessed by the "Settings" menu), there is a floppy disc icon to save settings. Note that print and printer settings are saved separately. If the drop-down names specify "modified' then they must be resaved.



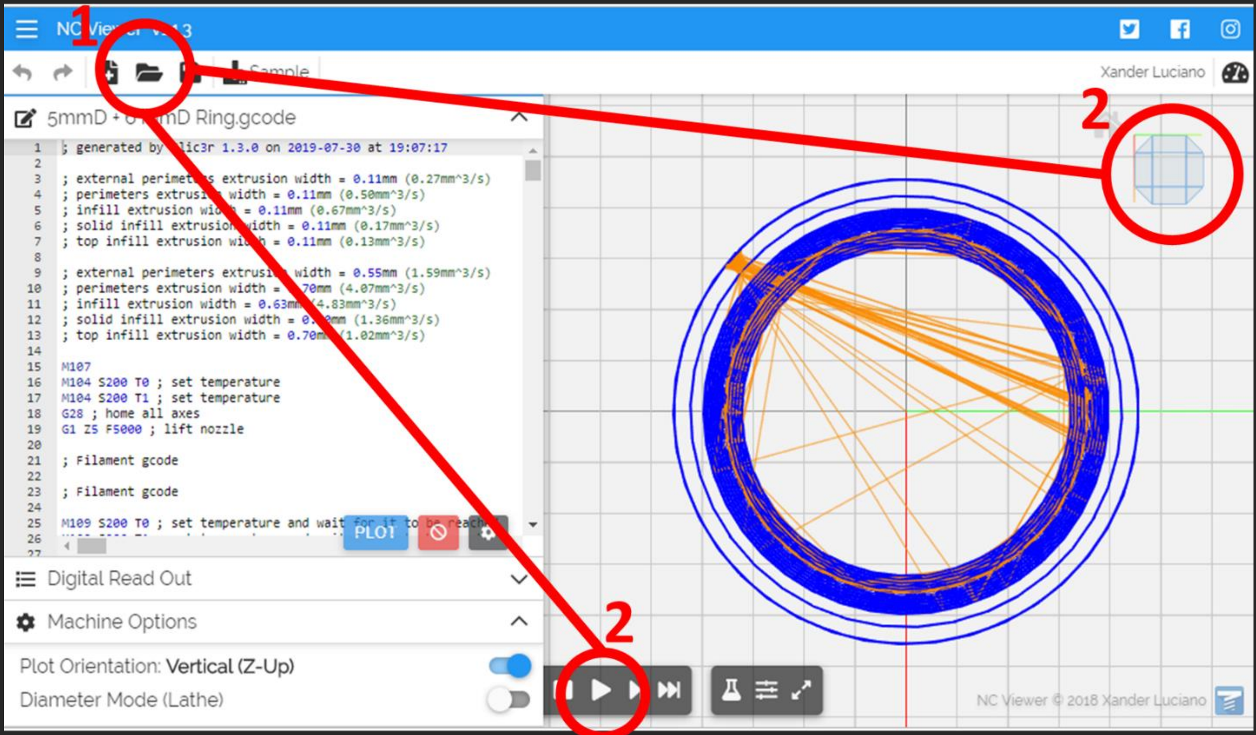
Generating G-code: After all the above steps are complete, go to the "Plater" tab. Set "Print Settings" and "Printer Settings" to the profiles that were saved earlier. Then hit the "Export G-code" button to generate the g-code file.



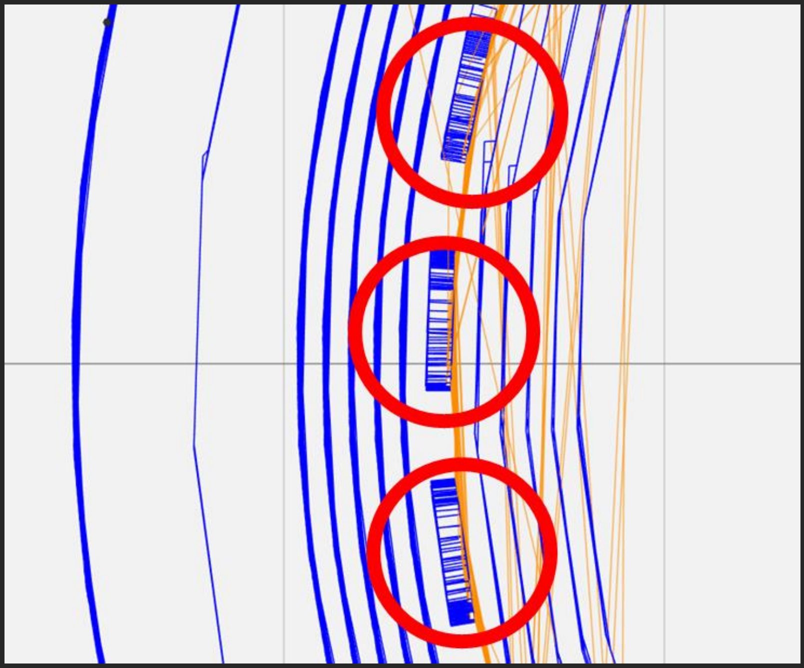
Viewing Tool Paths: Slic3r has a built-in tool path viewer but for this guide, NC Viewer will be the recommended g-code viewing tool. Click on the load button on the top left to import the g-code generated from Slic3r. press the play button at the bottom to see a how the machine progresses along the tool path. Orange lines represent movement only and blue lines represent printing. The specific line of G-code being executed with tool path progress is displayed to the left. In general, tool path viewers are great ways to visually check g-code for issues and to learn about how different slicing options affect the print.

Note that any g-code viewer, in the context of ModiPrint, only displays tool paths (the movement path of printheads). Print settings and other parameters are set in ModiPrint's desktop program which is further down in the workflow.

On the top right, the viewing angle can be set. By setting these concentric cylinders to a top-down view, slicing parameter effects on the tool path can be viewed. The tool path can be seen to fit nicely within the profile of the cylinder, a result of the concentric infill pattern setting. The outer cylinder was set with a larger XY spacing (500 µm) and the inner cylinder has a smaller XY spacing (100 µm) which is clearly seen in the top-down view. Z spacing, which is the same for both cylinders, can be seen with a side view.



Fine Tuning XY Spacing: Zoom into the tool path on NC Viewer.



Hmm, what are those? That happens when the slicing program cannot perfectly fill the geometry of the print with what the software perceives as the diameter of the filament. With very complex geometries, this may not be avoidable. However, cyclinders are not so complex and these defects can be optimized away. For a perfectly fitting tool path in this situation, the XY spacing must be perfectly divisible by the geometry. 0.1 mm and 0.5 mm for the two extruders can perfectly subdivide the 1 mm thickness of the cyclinders.

Unfortunately, spacing cannot be directly set as Slic3r is not designed for bioprinting and 100% infills. Being exact about the XY tool path spacing is difficult as the calculation is complex and not thoroughly documented. To be frank, it's not possible to entirely math this out without delving into the source code.

In Slic3r's calculations, the XY tool path spacing is dependent on extrusion width via this formula:

```
spacing = extrusion_width - layer_height * (1 - PI/4)
```

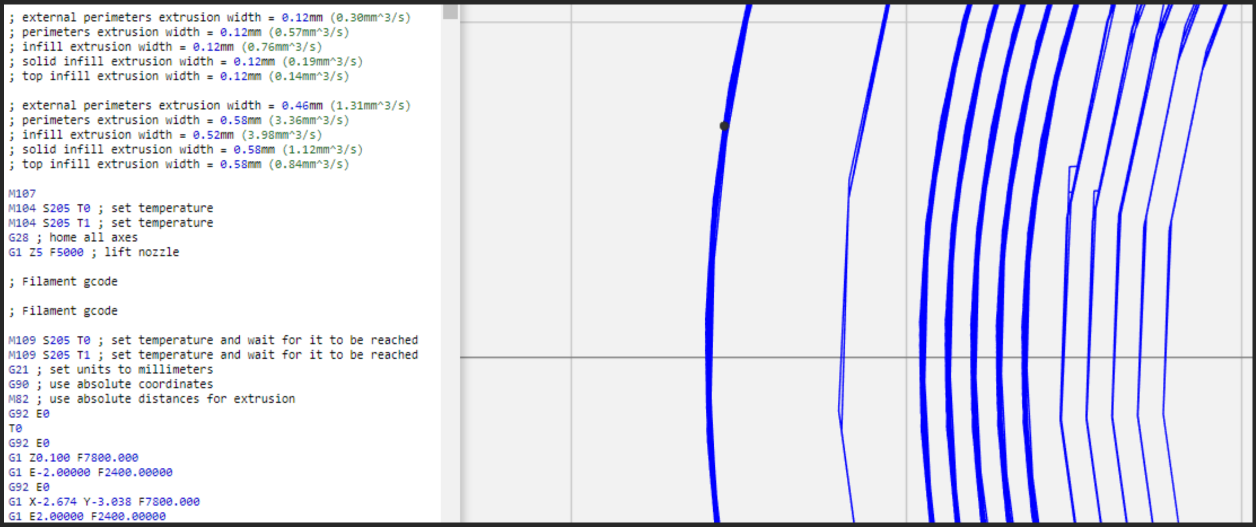
The example's layer height is 0.1 mm, so achieving a spacing of 0.1 mm and 0.5 mm takes extrusion widths of 0.12 mm and 0.52 mm respectively. Extrusion width is the perceived filament size which can be found at the beginning of the generated g-code file which can be viewed with Notepad.

```
; generated by Slic3r 1.3.0 on 2019-07-30 at 19:07:17

; external perimeters extrusion width = 0.11mm (0.27mm^3/s)
; perimeters extrusion width = 0.11mm (0.50mm^3/s)
; infill extrusion width = 0.11mm (0.67mm^3/s)
; solid infill extrusion width = 0.11mm (0.17mm^3/s)
; top infill extrusion width = 0.11mm (0.13mm^3/s)

; external perimeters extrusion width = 0.55mm (1.59mm^3/s)
; perimeters extrusion width = 0.70mm (4.07mm^3/s)
; infill extrusion width = 0.63mm (4.83mm^3/s)
; solid infill extrusion width = 0.70mm (1.36mm^3/s)
; top infill extrusion width = 0.70mm (1.02mm^3/s)
```

Notice that the extrusion widths are different for each section of the print. In the concentric cylinder example, perimeters and top layers are nonexistent. Slic3r will output extrusion width parameters for all sections of the print regardless of whether they are relevant. The only parameters relevant to the print is the "infill extrusion width".

To fine tune extrusion width, change either the speed settings (under "Print Settings" → "Speed") or the nozzle diameter. The nozzle diameter must be empirically fine-tuned until infill extrusion widths of 0.12 mm and 0.52 mm are achieved. This is done with the process of iterating nozzle diameter parameters, exporting g-code, and viewing the g-code file until we hit our desired targets. For this example, the correct nozzle diameters are 0.11 mm and 0.415 mm.

On NCViewer, the new cyclindrical toolpaths are very clean. The inner cylinder is subdivided into exactly 10 concentric passes with 0.1 mm spacing. Our outer cylinder is exactly 2 concentric passes with 0.5 mm spacing.



This raw g-code file of this example is located in the “Tool Path Samples” folder, named “5&6mm Concentric Cyclinders.gcode”.

Additional Resources

This guide has only touched the surface of all Slic3r’s features. For more information see the Slic3r manual. If Slic3r does not provide a specific feature, consider using other slicing software such as Cura. Alternatively, users ca modify g-code tool paths or the generated ModiPrint commands themselves.

Generating G-code by Hand

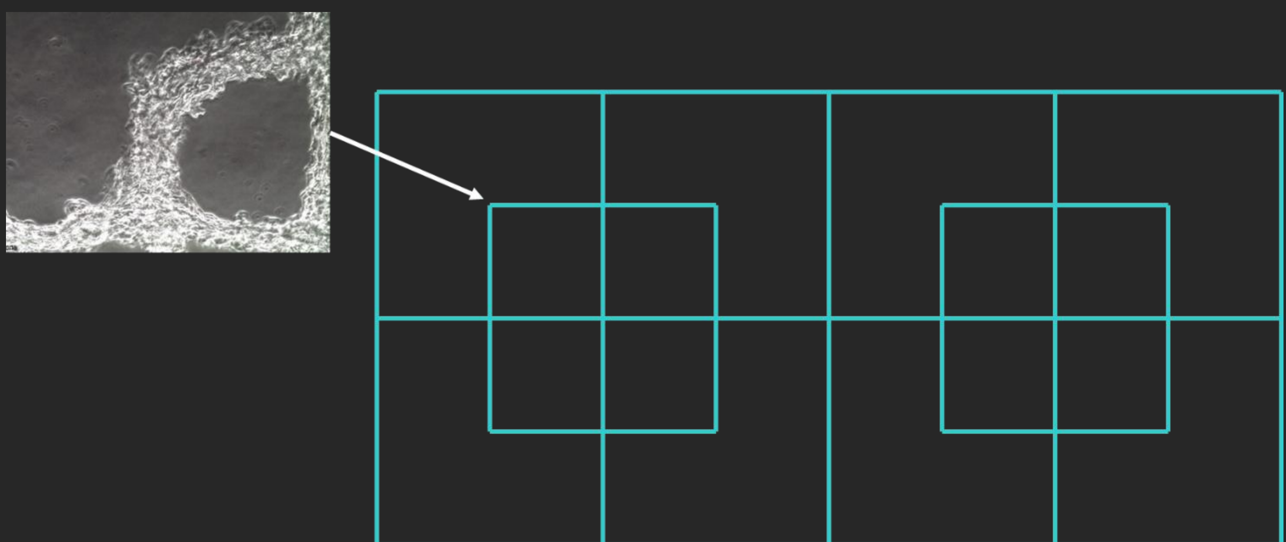
If ModiPrint is not being used to print complex geometries from CAD files, or if the shape is not sliceable, then consider generating g-code by hand. See ModiPrint's workflow and generating g-code via CAD files before deciding to write a g-code file from scratch.

Unlike slicing, the process is very basic. Simply:

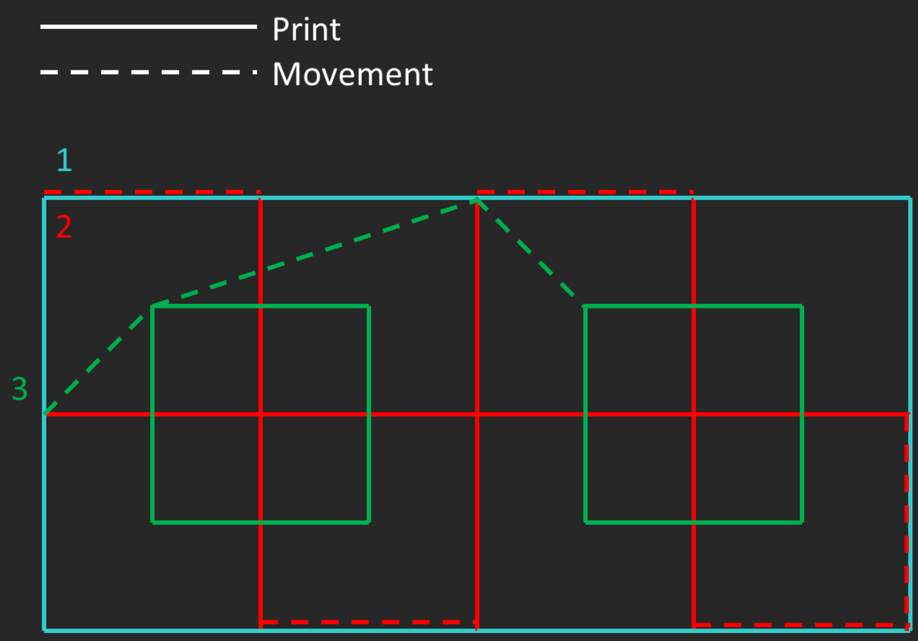
1. Create a file with a text editor, such as Notepad.
2. Write ModiPrint-supported g-code in the text file.
3. Change the file extension to .gcode.

To perform step 2, study the ModiPrint-supported g-code to see what commands are accepted by ModiPrint's desktop program.

This section will example step 2 by generating a .gcode file that prints a single filament thickness shape that is diagramed below.



This was the same geometry used to create the corners in Figure 3C-E of ModiPrint's paper. TO DO It is a simple shape that isolates right angled filaments for imaging while maintaining the support necessary to hold the geometry intact. As slicing software does not provide this type of pattern, the g-code for this geometry was necessarily written by hand. The steps to create such a geometry is detailed below.



The first step is to generate the outer rectangle. Then the tool path weaves the structural supports within the rectangle. Finally, the tool path moves through the structural supports to create the exposed 90-degree angles.

Begin the g-code file with the following line:

T0 ; Set the material that will be used for future actions

Anything that appears in the same line after a semicolon will not be read by ModiPrint. The text after a semicolon is a comment and is intended for user annotation. The first line, "T0", sets the material and all associated material settings. Without the "T0" line and a material with the "T0" g-code ID, no movement or printing will occur.

Assuming the outer rectangle is 8 mm (width) x 4 mm (length), we will then write the following line:

G91 ; Set future coordinate values to relative position
GX X8 ; Print a line 8 mm in the positive X direction
GX Y-4 ; Print a line 4 mm in the negative Y direction
GX X-8
GX Y4

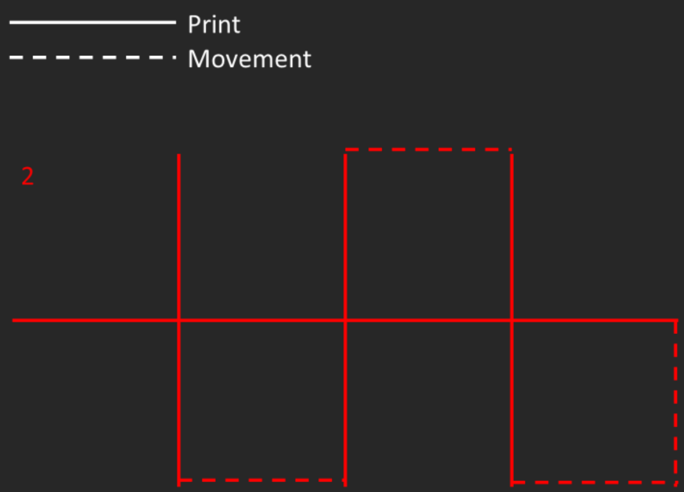
The rectangle can also be printed with absolute position commands as such:

G90
GX X8
GX X8 Y-4
GX X0 Y-4
GX X0 Y0

"G91" sets the system to read relative positions. The system always reads position values in millimeter units regardless of command. The "GX" command is a printing with movement command. "GX X8" prints an 8 mm line in the X direction. The three GX commands following complete the other sides of the rectangle.

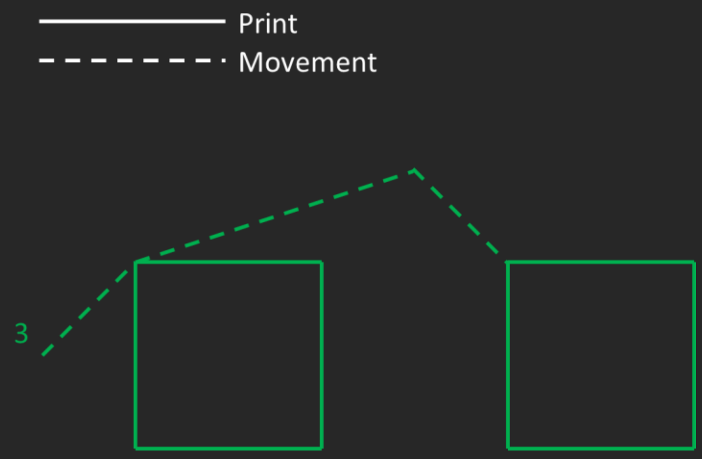
Next, draw the lines that weave in between the rectangle to form the supporting structure (shown in red in the figure). However, this cannot be drawn in a single, continuous line. Therefore, pure movement commands (G00) must be weaved in between print commands (GX).

```
; The system is still reading values in relative position
G00 X2 ; Moves 2 mm in the positive X direction without printing
GX Y-4 ; Print a line 4 mm in the negative Y direction
G00 X2
GX Y4
G00 X2
GX Y-4
G00 X2
G00 Y2
GX X-8
```



Finally, draw the boxes that weave between each support. This pattern was intended to be printed in a support bath, which is why the pure movement sequences move diagonally to avoid tracing back over and disturbing the integrity of the 90-degree corners.

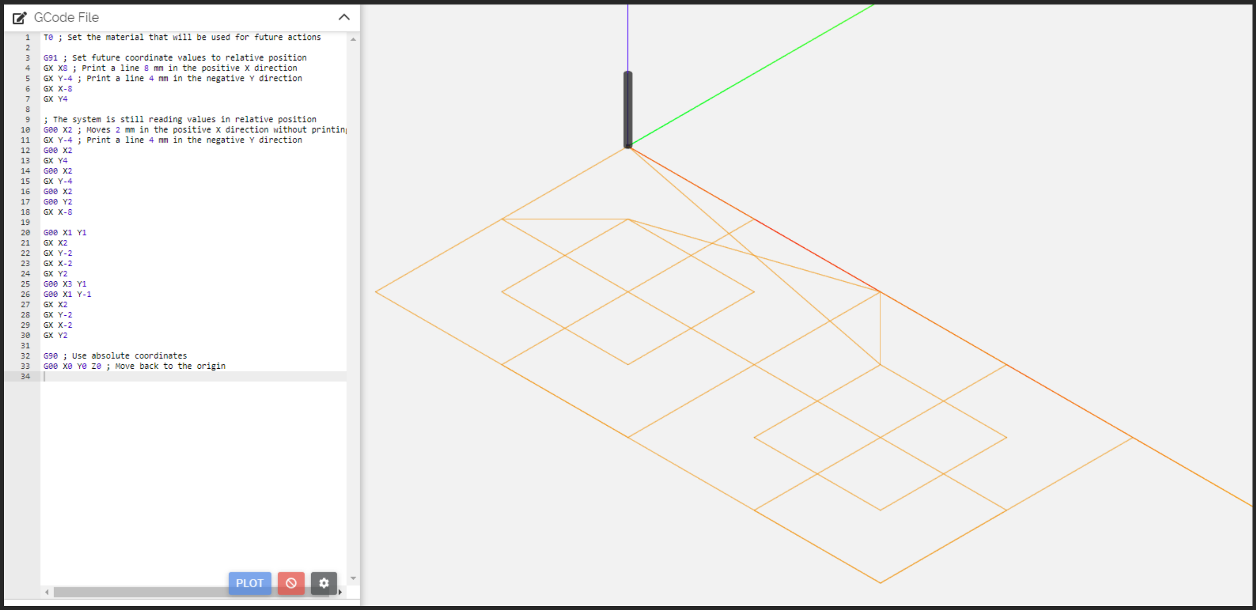
```
G00 X1 Y1
GX X2
GX Y-2
GX X-2
GX Y2
G00 X3 Y1
G00 X1 Y-1
GX X2
GX Y-2
GX X-2
GX Y2
```



To reset the printer back to origin after the print, append:

```
G90 ; Use absolute coordinates
G00 X0 Y0 Z0 ; Move back to the origin
```

GX commands and handwritten g-code also work with NCViewer. However, GX is read as movement instead or printing. Putting the entire example looks like this:



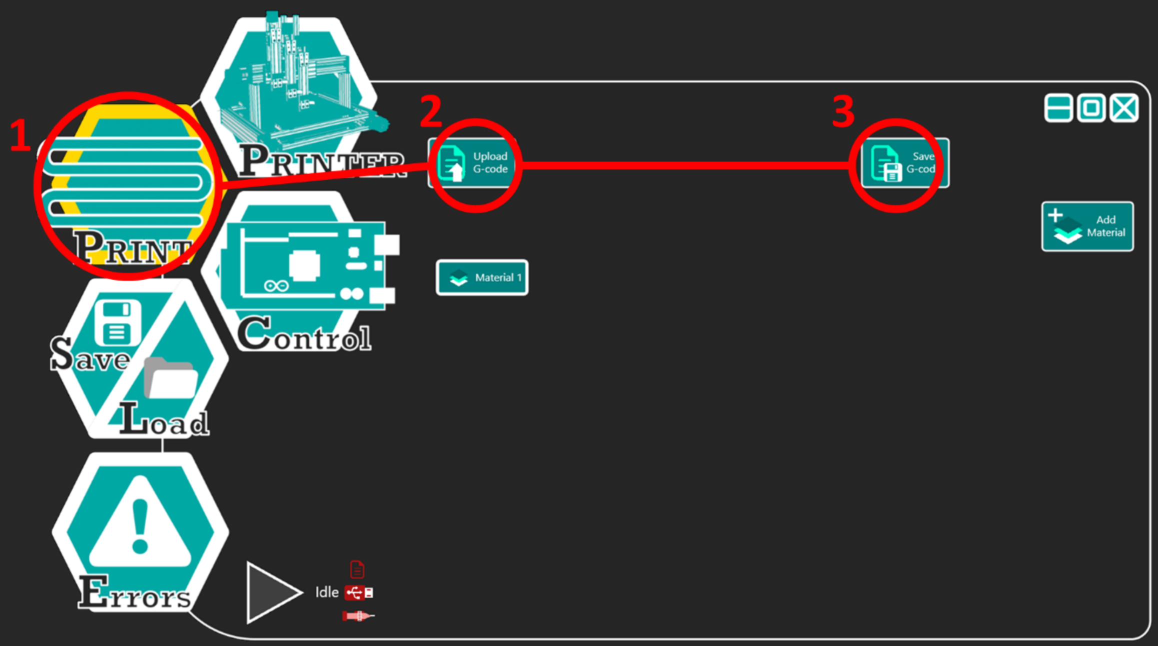
After generating the g-code file, simply upload the .gcode file in ModiPrint's desktop program just like any other g-code file.

Print Settings

The “Print” menu in ModiPrint’s desktop program controls which tool path (via a g-code file) is used and how the printer will execute that tool path. Settings under this menu will affect the execution of a print sequence. Before setting parameters in the “Print” menu, all parameters in the “Printer” menu must be set first.

G-code & ModiPrint

Hit the “Upload G-code” button and select the .gcode file that was generated from slicing software. If uploaded successfully, the file name will appear to the right of the “Upload G-code” button. To convert the g-code to ModiPrint’s language then save the resultant .mdpt file, set the material settings then hit the “Save G-code” button and select the appropriate destinate path. If a .mdpt file is selected during “Upload G-code”, then material settings do not need to be set before printing.

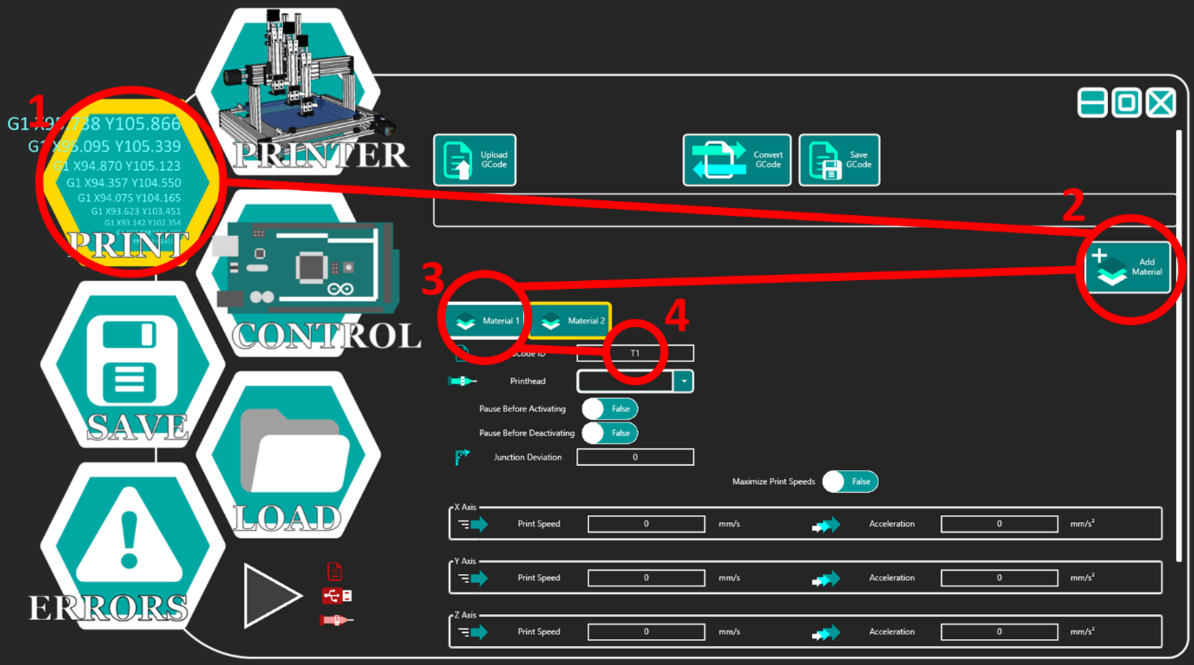


Multiple Materials

Each tool head is represented by a “T#” line in the .gcode file. Multiple tool heads would be in the .gcode file if the file was generated with multiple extruders in slicing software.

Each tool head in the g-code file corresponds to a different material in ModiPrint. ModiPrint will assign different print parameters of different speeds, printheads, print styles, etc. to the tool path following each "T#" command.

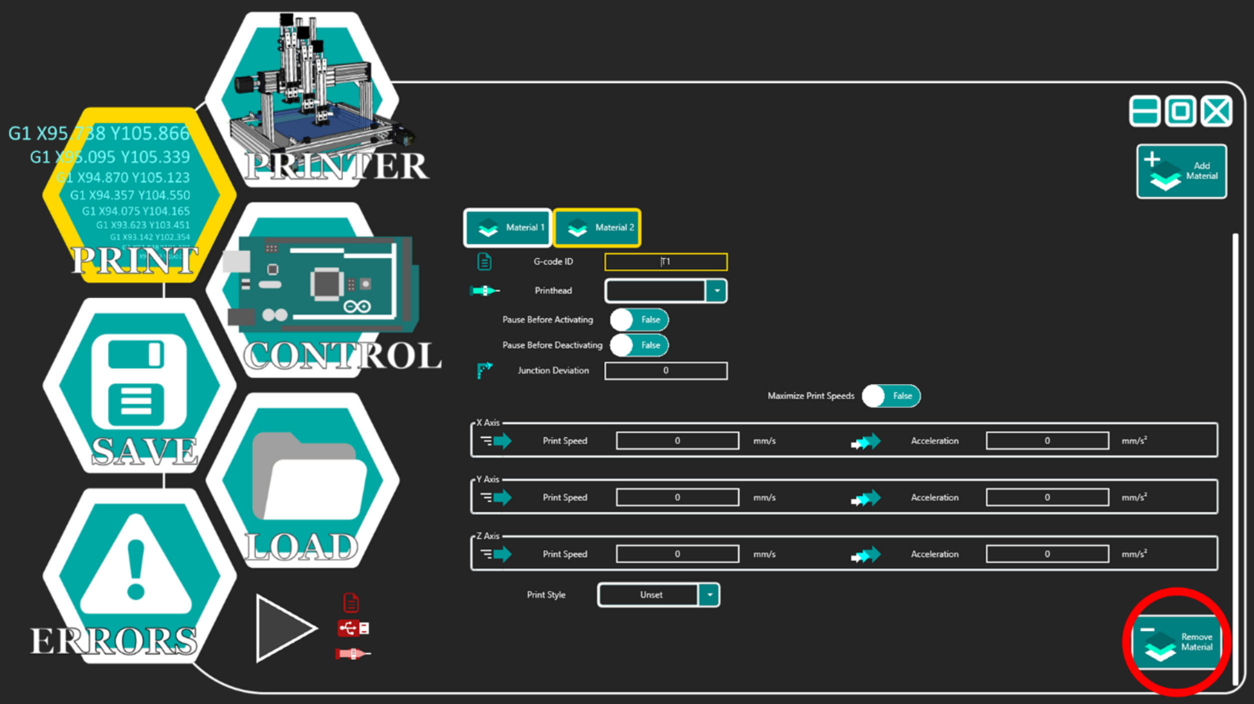
To set multiple materials in ModiPrint's desktop program, click "Add Material" for each tool head beyond the first. Each "Material #" tab corresponds to a different "T#" g-code line. Ensure that each "T#" line in the g-code file is represented by a unique g-code ID parameter in ModiPrint.



The example from the Generating G-code section of this guide generated a g-code file of two concentric cylinders, each produced by different tool heads. The inner cylinder was assigned to extruder 1 which corresponds to the g-code command "T0". Extruder 2 corresponds to "T1". In Slic3r, extruder X will correspond to "T(X-1)", so extruder 3 would be "T2", extruder would be "T3", etc. Therefore, ModiPrint's "Material 1", with its g-code ID set to T0, would correspond to extruder 1 and "Material 2", with its g-code ID set to "T1", would correspond to with extruder 2.

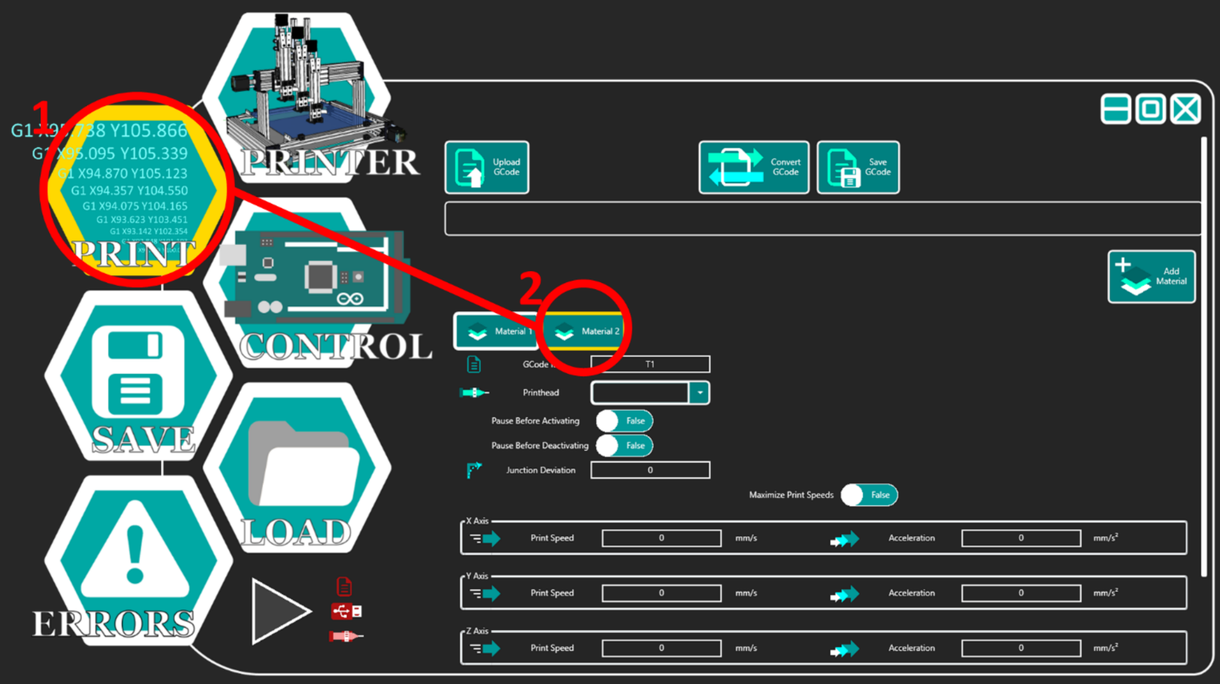
Note that if there is a new tool head command in the g-code and no material with a corresponding g-code ID is set, then g-code conversion will fail and produce an error message. It is not recommended that multiple of the same g-code ID is set across multiple materials. But if this case occurs, then the earliest created material with that g-code ID will be used.

To remove a specific material, navigate to the tab corresponding to that material then the "Remove Material" button (scroll down to see it). Note that deleted materials will not remember its settings.



Material Settings

Click on the appropriate material tab to set to parameters for that material.



G-code ID: This material will correspond to this g-code ID in the g-code file. When the program reads this value in the g-code file, it will use the settings of this material to execute subsequent actions. The g-code ID values will only appear in the dropdown after a g-code file has been uploaded. If a g-code ID is not displayed in the dropdown, then the g-code file does not contain this value of that same g-code ID is set to another material. The same g-code ID cannot be set to multiple materials. Clicking the X to the right of the g-code dropdown will clear the selection and allow use of that g-code ID elsewhere.

Printhead: This is the printhead that will be used with this material. The printhead and its parameters should be set in the "Printer" menu before this parameter is set. Multiple materials can set the same printhead. This will allow the same printhead to execute different sections of the print with different parameters.

Pause After Activating/Before Deactivating: If "Pause After Activating" is set to true, then a pause in the print sequence will occur after switching to this material. If "Pause Before Deactivating" is set to true, then then a pause in the print sequence will occur before switching away from this material. During this pause sequence, other operations can occur via the "Control" menu. For example, this option can be set to true if the material reservoir needs to be changed before switching materials. Or maybe a printhead needs to be unclogged before use. During the pause state, the print sequence will only resume after the "Resume" button is pressed.

Junction Deviation: Determines the centripetal acceleration of the XYZ stage which allows actuators to skip the initial stages of acceleration. Must be set to a value between 0 and 1 where 0 has each line segment starts at zero velocity. A higher junction deviation enables faster junction speeds which results in more constant speed overall. Therefore, a higher junction deviation is beneficial for better maintaining a consistent filament diameter during continuous extrusion printing. This parameter should be set as high as possible considering that the XYZ stage frame is stiff enough to handle the jerk. A junction deviation that is too high will result in imprecise prints. The recommended setting for ModiPrint hardware is 0.01.

Maximize Print Speeds: If toggled to true, then all print speeds will be set to the maximum values as they are set under the "Printer" menu.

Print Speed (mm/s): This is the speed at which the actuator will move during printing in mm/s. Higher speeds will reduce operation time. For continuous extrusion printing, higher print speeds will decrease filament diameter. The X and Y actuators shares the same print speed value. The Z actuator uses a different print speed value.

Acceleration (mm/s^2): Acceleration that the actuator will move at during printing in mm/s^2. Typically, there is no reason why this shouldn't be set to the maximum acceleration value as set in the Printer menu. The X and Y actuators shares the same print acceleration value. The Z actuator uses a different acceleration value.

Print Style: If set to continuous print, the printhead will continually dispense as the XYZ stage is moving. If set to droplet print, the printhead will stop at regular intervals along the tool path and dispense specified volumes for these droplets. Leaving this value as unset will produce errors while converting g-code. Different print style settings and printhead types will change the parameters that appear on the interface.

Continuous Print Style

Valve Printhead Type – Continuous Print Style: There are no parameters to set for this combination of printhead type and print style. Simply, the valve opens and pressure drives dispensing only when the printhead is moving through the tool path.

Motor-driven Printhead Type – Continuous Print Style – Dispense Distance Per mm Movement (mm): This parameter determines the distance that the motor-driven printhead will traverse in relation to the distance of the tool path in mm. For example, if this parameter is set to 0.02 mm and the bioprinter is to print a 100 mm line, then the motor-driven printhead will move 2 mm during that operation.

In theory, the printed filament's geometry follows this formula:

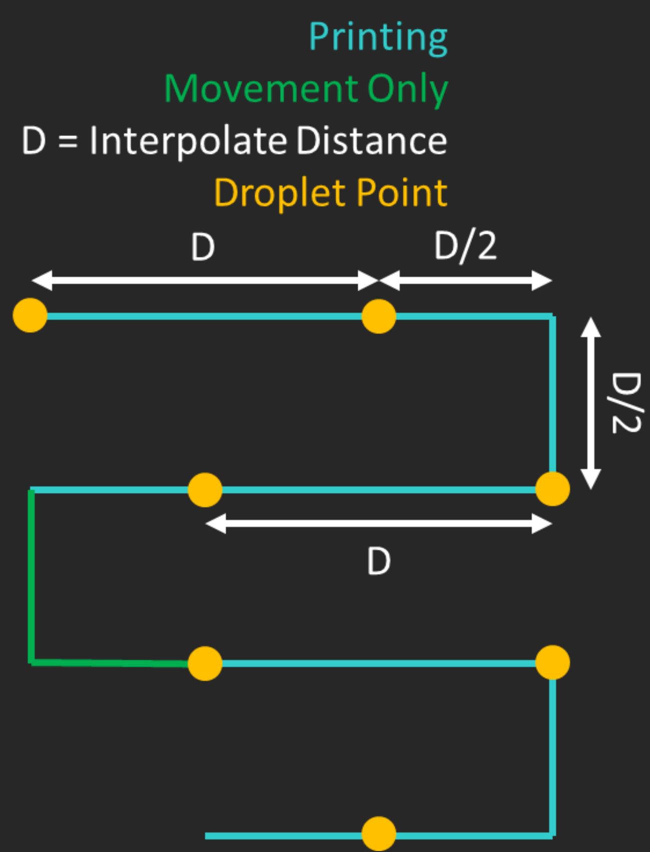
$$Filament\ Cross\_sectional\ Area = Dispense\ Distance\ Per\ mm\ Movement * Resevoir\ Cross\_sectional\ Area$$

Where the reservoir cross-sectional area would be, for example, the radius of a syringe barrel * πr^2.

Motor-driven Printhead Type – Continuous Print Style – Retraction Distance (mm): This parameter determines the distance that the motor-driven printhead will traverse when retracting in mm. Retraction moves the motor-driven printhead in the direction that is opposite to dispensing. This action draws the material away from the nozzle and prevents excess material deposition. The bioprinter will remain in a retracted state whenever the motor-driven printhead is not dispensing.

Droplet Print Style

The diagram below describes how ModiPrint's droplet printing parameters interacts with the tool paths provided by g-code.



At the beginning of any tool path in droplet mode, the printhead will dispense a droplet. At intervals of every D distance, as defined in the parameter "Interpolate Distance", another droplet will dispense. Notice that if a line segment ends before the entirety of the interpolate distance is travelled, the remainder of the interpolate distance carries over to the next line segment. If there is no line segment for the remainder to carry over, then no more droplets will be print. This process is reset if movement without printing occurs.

ModiPrint's droplet printing feature only determines droplet spacing, not tool path spacing (tool path spacing is D/2 in this example). Tool path spacing in any ModiPrint feature is determined entirely by g-code.

Valve Printhead Type – Droplet Print Style – Valve Open Time (µs): Each droplet will be dispensed via opening the valve printhead for this duration of time in µs. With default hardware using the VHS solenoid valves, minimum open time value is ~100 us.

Motor-driven Printhead Type – Droplet Print Style – Dispense Distance (mm): Each droplet will be dispensed via this traverse of the motor-driven printhead in mm. Higher dispense distance values will result in higher volume droplets.

Motor-driven Printhead Type – Droplet Print Style – Dispense Max Speed (mm/s): Maximum speed that this actuator can traverse during dispensing of droplets in mm/s. Higher values will cause more force to be applied for shorter durations to the ink, but the droplet volume should (in theory) not change.

Motor-driven Printhead Type – Droplet Print Style – Dispense Acceleration (mm/s^2): Maximum acceleration that this actuator can traverse during dispensing of each droplet in mm/s^2. Typically, there's no reason why this shouldn't be set to the maximum acceleration value as set in the Printer menu.

Interpolate Distance: Distance between each droplet.

Concentration Gradient

Droplet Print Style – Concentration Gradient – Gradient Shape: ModiPrint can generate concentration gradients by varying the dispense volume during droplet printing. To disable this feature, set the gradient shape parameter to none. Otherwise, this parameter determines the shape that the concentration gradient radiates from. Point would produce a spherical gradient, line a cylindrical gradient, and plane a rectangular gradient.

Droplet Print Style – Concentration Gradient – Gradient Strength: The dispense volume during droplet printing is determined by this gradient strength parameter and the distance of the droplet from the gradient shape and its coordinates. Specifically, this feature varies the dispense volume by varying valve open time for valve printheads or dispense distance for motor-driven printheads. The formulas for linear and exponential gradients are as follows:

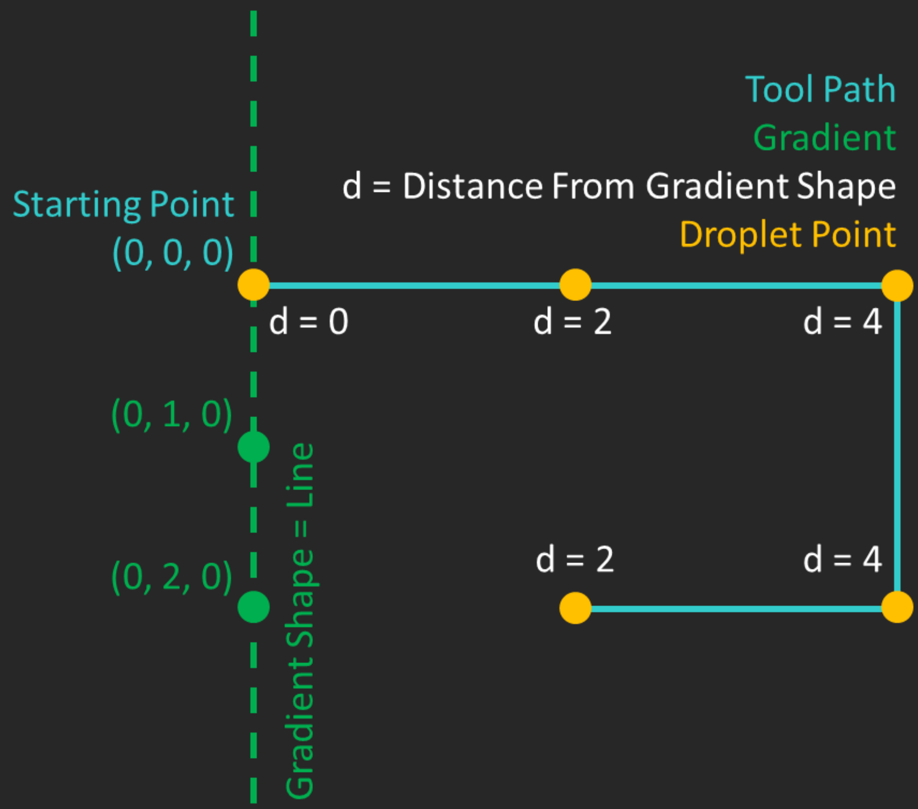
Linear Formula: $Dispense\ Distance_d = Dispense\ Distance_0 * (1 + Gradient\ Strength * d)$

Exponential Formula: $Valve\ Open\ Time_d = Valve\ Open\ Time_0 * (1 + Gradient\ Strength)^d$

Where *Print Parameter*$_d$ is either the “Valve Open Time” or “Dispense Distance” parameter at some distance *d* from the gradient shape. *Print Parameter*$_0$ is the print parameter at $d = 0$ (origin), which is equivalent to the “Valve Open Time” or “Dispense Distance” parameter. *Gradient Strength* corresponds to the percent value specified by the “Gradient Strength” parameter. Higher *Gradient Strength* results in steeper gradients.

Droplet Print Style – Concentration Gradient – Coordinate (mm): Determines the location of the gradient shape in mm. (X0, Y0, Z0) is the starting location of this material and any other coordinate is some mm relative to this origin. A point gradient will require one coordinate, a line requires two, and a plane requires three. All coordinates will be located within their corresponding shape. Therefore, the same coordinate shape can be set with different sets of coordinates. If more than one coordinate has the same position values, an error will occur.

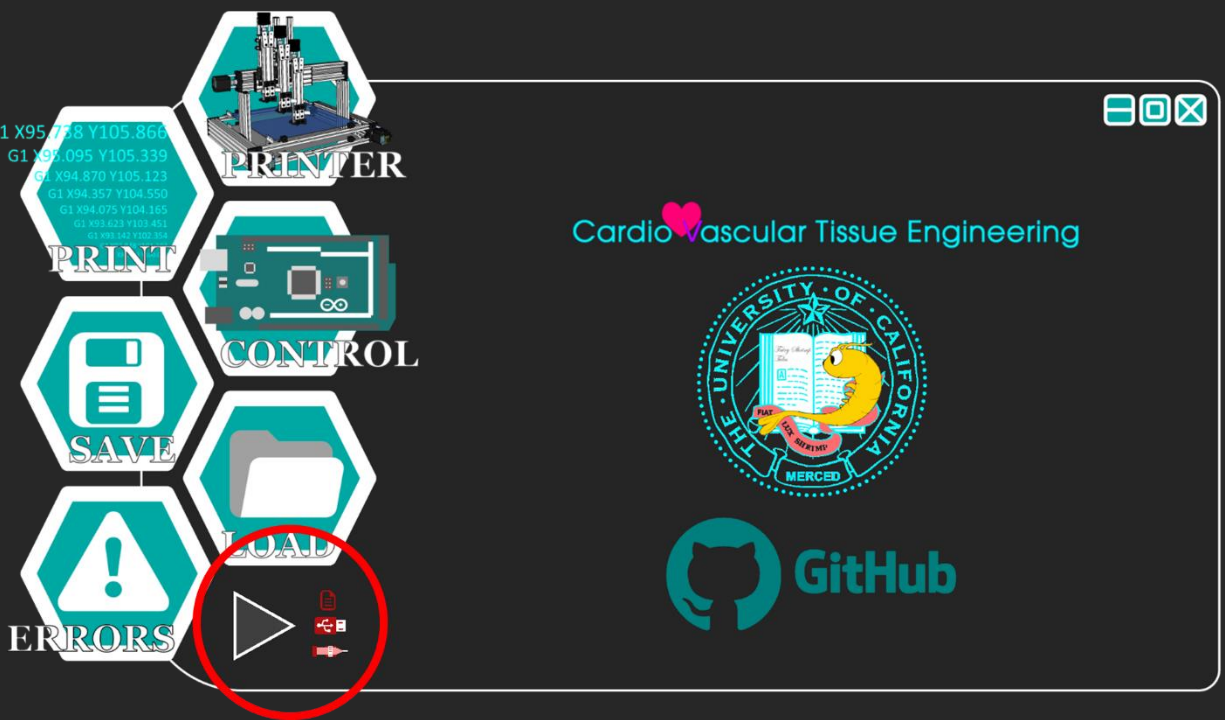
See the picture example below. A gradient shape of a line with coordinates (0, 1, 0) and (0, 2, 0) will generate a line that intersects the origin and is parallel to the Y axis. The printhead will follow the tool path and produce droplet sizes according to a cylindrical gradient around the gradient line.


Tool Path
Gradient
d = Distance From Gradient Shape
Droplet Point
Starting Point
(0, 0, 0)
d = 0
d = 2
d = 4
(0, 1, 0)
(0, 2, 0)
Gradient Shape = Line
d = 2
d = 4

Print Sequence

Executing a print sequence will stream the ModiPrint language from the desktop program to the microcontroller. This is the last step in the workflow that translates CAD models to g-code to ModiPrint operation.

From any menu, the interface for executing a print sequence can be seen on the bottom left.



Requirements: When initially starting the program, the print button (triangle) will be greyed out, meaning the software is not yet set to print. Certain requirements, represented in the icons to the right of the print button, will need to be met before printing can occur. As these requirements are met, these icons will change from red to turquoise. When all three requirements are met, the print button will change to turquoise and can be clicked to begin a print sequence. The three requirements are:

Establish a serial connection to the microcontroller.

Upload a .gcode or .mdpt file. A .gcode file will be converted to a .mdpt file and data from this .mdpt file will be processed then streamed during the print sequence. Additionally, each tool head (T#) command from the .gcode file must be set as a RepRapID parameter in under Materials in Print Settings.

Set a printhead. The print sequence will begin with the dispensing location of the printhead as the origin. Therefore, maneuver the printhead to the starting location of the print before executing the print.

Execution: When all requirements are set, begin the print sequence by hitting the turquoise play button. A print sequence will stream commands from converted g-code file directly to the microcontroller by looping the following procedure:

1. ModiPrint's desktop program will send a command to the microcontroller.
2. The microcontroller program will reply that it has received the command.
3. In a separate reply, the microcontroller program will report that it is ready to receive another command.

At the very end of the procedure, ModiPrint will append a command to retract the active Z actuator back to its default position.

This process can be interrupted if the print sequence button is pressed or by interrupts from the hardware. The print sequence button icon and purpose changes with the status of the print sequence.

This button indicates that no print sequence is occurring, and parameters are not set to execute the sequence. This button cannot be pressed.

This button indicates that no print sequence is occurring, but the program is ready to execute the sequence. Pressing this button will begin the print sequence.

This button indicates that a print sequence is occurring normally. This button can be pressed to pause the print sequence. Pausing will occur at the next available point that does not interfere with the print. This may mean that many movement and print commands with cornering speed parameters be executed before the printer pauses. While paused in this manner, manual commands can be executed.

This button indicates that the print sequence is paused. Manual commands can be executed while paused. Pressing this button will resume the print sequence.

This button indicates that the print sequence is paused, and the microcontroller has ceased operation. This occurs without user intervention when a limit switch is unexpectedly hit. Manual commands cannot be executed in this mode as the microcontroller's protocol has been disturbed. Although this button can be pressed to resume the print sequence, the position of the XYZ stage is most likely incorrect and the print is unsalvageable. It is recommended that users reset the software and hardware and fix the out of range issues before restarting the print sequence.

Abort: The abort button is located right above the print sequence button. After pressing the abort button, an additional menu will prompt users to press abort again or press return to cancel the abort process.

During a print sequence, pressing the abort button twice will stop the print sequence as soon as possible. This is different from the pause button in that aborting will completely abandon the print sequence and stop hardware operations immediately. This is an abnormal termination of protocol and will require a restart of the entire print sequence. Synchronization error messages may occur.

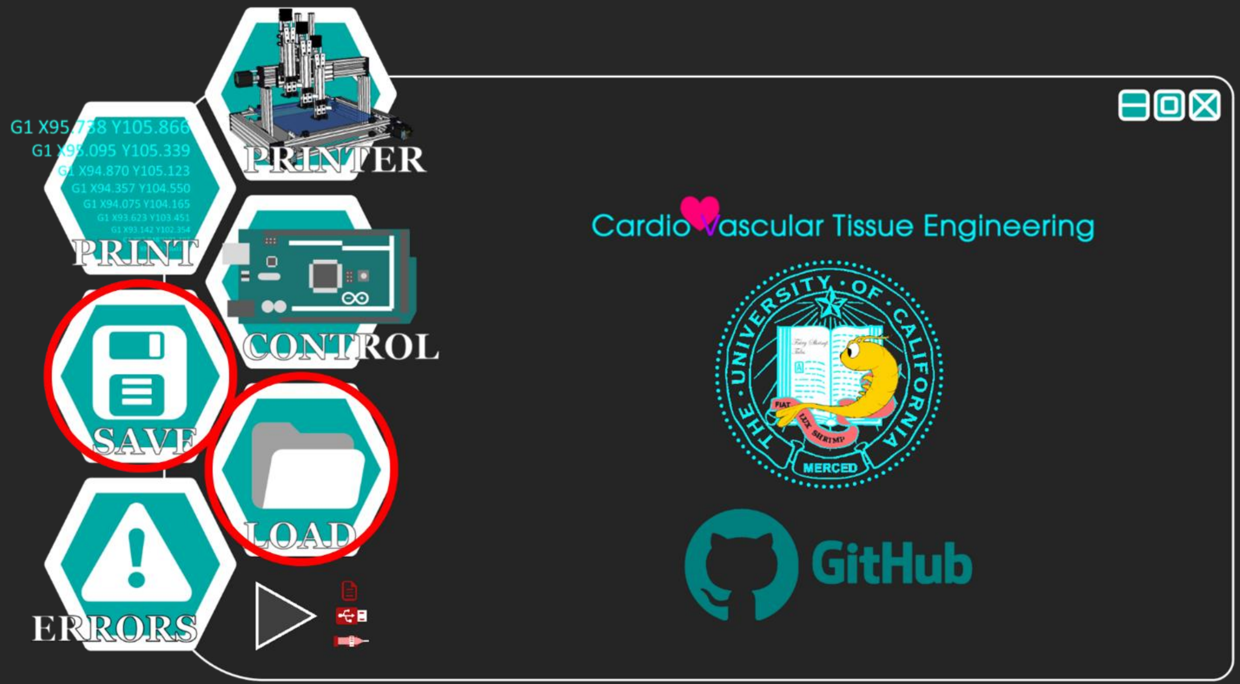
Outside of a print sequence, the abort button will reset the microcontroller and all set equipment on the desktop program. This is useful if the program is ever stuck because of any reason. If the program is ever non-operational after aborting, restart the microcontroller by cutting power and restart the desktop program.

Pressing the back button will cancel the abort process.

Saving and Loading

Saving stores all information set in the “Printer” and “Print” menus into a .xml file. Therefore, printer and print parameters should be set first before saving. The load feature will retrieve all printer and print parameters from the XML file. Editing the XML file might cause errors while loading.

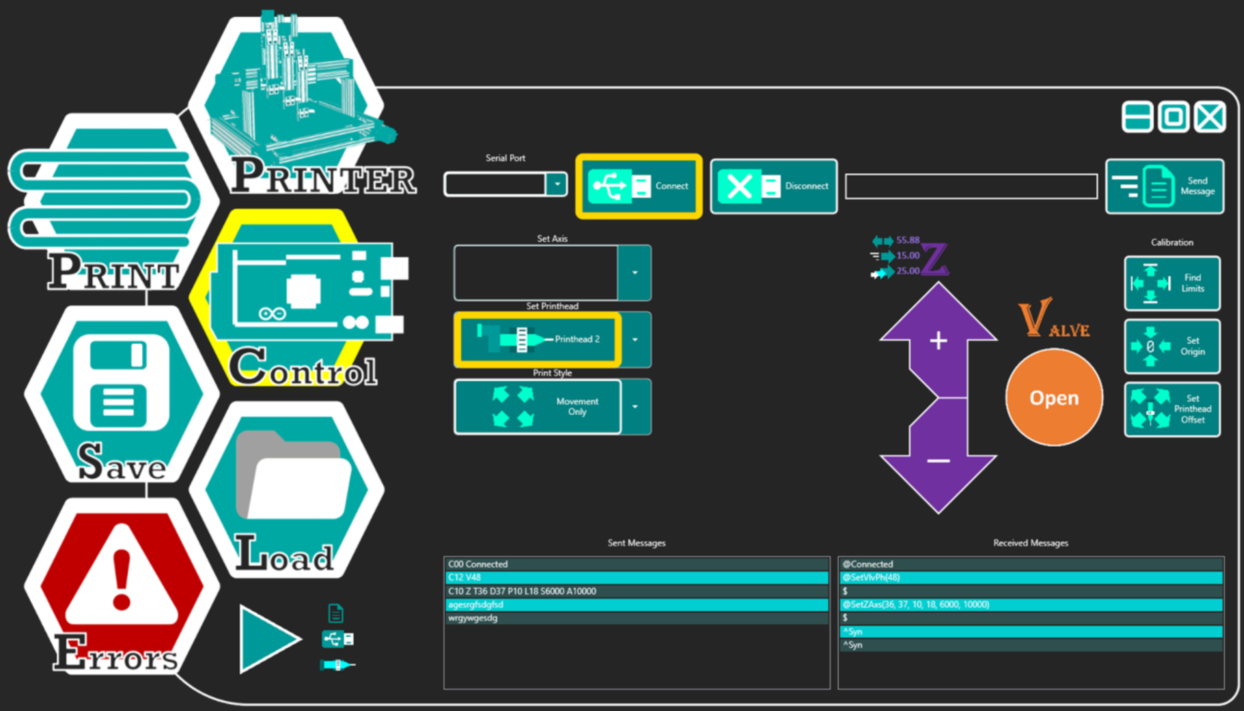
To save ModiPrint settings, click on the “Save” hexagon and choose the destination path. To load ModiPrint settings, click on the “Load” hexagon and choose the appropriate XML file.



Errors

When an error occurs with any of ModiPrint's processes, the "Error" hexagon will turn red. Click on the "Error" hexagon to view all errors that have occurred.

The left column displays which process the error occurred at. The right column displays what the error is. An error typically occurs because actions were performed out of sequence. Contact us if errors persist.



In the example above, a nonsensical message was sent through the serial port. The microcontroller replied with "^Syn", which signifies a syntax error. The error hexagon turns red, which indicates that there is a new error. Clicking on the error hexagon will display the errors list.

ModiPrint Commands Documentation

The following documents the language used to communication with ModiPrint's microcontroller program. Understanding these commands is typically unnecessary. This is only required for manually editing .gcode, editing .mdpt files, or manually sending messages to the microcontroller.

This documentation will have the following format:

Command: The raw message queued by ModiPrint's desktop program when executing an action. Commands can be further modified by ModiPrint's desktop program before being sent to the microcontroller. Unless specified, all commands can be directly sent to the microcontroller via serial port.
Actions: What occurs after this command is processed and executed.
Parameters: What parameters can be modified on this command.
Return: The message that is returned by the microcontroller.

C Commands: Calibration and Setup

C00: Check Connection

Command: C00 *;
Actions: Returns message after command.
Parameters: * sets the message to be returned.
Return: *

Example:
Message: C00 Connected;
Actions: Nothing.
Return: Connected

C10: Set Axis

Command: C10 X/Y/Z T# D# P# L# S# A#;
Actions: Sets the parameters for an axis. All future axis operations will use these parameters until future C10 commands override them.
Parameters: First letter sets the X, Y, or Z axis.
T# sets the GPIO Pin signaling the step of the motor. 54-70 for Analog Pins A0-A15 respectively. 0-53 for all others.
D# sets the GPIO Pin signaling the direction of the motor.
P# sets the step pulse duration (microseconds).
L# sets the GPIO Pin receiving from the limit switches (maximum and minimum range limit switches share the same GPIO Pin). If # = 70, then no limit switch is set.
S# sets the max speed of the motor (steps/s).

A# sets the max acceleration of the motor (steps/s^2).
Returns: @SetYAxs(#, #, #, #, #, #, #);

Example:
Command: C10 Y T40 D41 P20 L70 S500 A100;
Actions: For future commands regarding Y Axis movement (such as G00 commands), the printer will drive the actuator motor with pins 40 (step) and 41 (direction) with a step pulse duration of 20 microseconds at a max speed of 500 steps/s from an acceleration of 100 steps/s^2. There is no limit switch to signal if this axis actuator moves beyond its maximum or minimum range. Future C01 Y commands will override the parameters set by this command.
Return: @SetYAxs(40, 41, 20, 70, 500, 100);

C11: Set Motor-driven Printhead

Command: C11 T# D# P# L# S# A#;

Actions: Sets the parameters for a motor-driven printhead. All future printhead operations will use these parameters until future C11/12/13 commands override them.
Parameters: First letter sets the X, Y, or Z axis.
T# sets the GPIO Pin signaling the step of the motor. 54-70 for Analog Pins A0-A15 respectively. 0-53 for all others.
D# sets the GPIO Pin signaling the direction of the motor.
P# sets the step pulse duration (microseconds).
L# sets the GPIO Pin receiving from the limit switches (maximum and minimum range limit switches share the same GPIO Pin). If # = 70, then no limit switch is set.
S# sets the max speed of the motor (steps/s).
A# sets the max acceleration of the motor (steps/s^2).
Return: @SetMtrPh(#, #, #, #, #, #);

Example:
Command: C11 T20 D21 P40 L41 S5000 A1000;
Actions: For future commands regarding printing, the printer will drive the motor-driven printhead's motor with pins 20 (step) and 41 (direction) with a step pulse duration of 40 microseconds at a max speed of 5000 steps/s from an acceleration of 1000 steps/s^2. A high voltage to Pin 41 will signal that this motor printhead's actuator has moved to its maximum or minimum range. This printhead's operation is not limited by limit switches.
Return: @SetMtrPh(20, 21, 40, 41, 5000, 1000);

C12: Set Valve Printhead

Command: C12 V#;
Actions: Sets the parameters for a valve printhead. All future printhead operations will use these parameters until future C11/12/13 commands override them.

Parameters: V# sets the GPIO Pin signaling the opening of the valve.
Return: @SetVlvPh(#);

Example:
Command: C12 V36;
Actions: For future commands regarding printing, the printer will operate the valve printhead with pin 36 signaling the valve.
Return: @SetVlvPh(36);

G Commands: Movement and Printing

G00: Movement

Command: G00 X# Y# Z# T#;
Actions: Moves up to three actuators. Speed and acceleration are matched such that all three XYZ stage axes start and stop virtually simultaneously.
Parameters: X# relative movement of the X axis (steps).
Y# relative movement of the Y axis (steps).
Z# relative movement of the Z axis (steps).
T# exit speed of the movement (steps / s). If this value is unspecified, then then exit speed is zero (complete stop at end of movement).
Return: @Mv(#, #, #);
There is no return value for exit speed.

Example:
Command: G00 X2 Y-3 Z4;
Actions: Move 2 steps in the X direction, -3 steps in Y, 4 steps in the Z.
Return: @Mv(2, -3, 4);

G01: Motor-driven Printhead Print with Movement

Command: G01 E# X# Y# Z# T#;
Actions: Moves the motor-driven printhead's motor. Also moves the three axes actuators. Speed and acceleration are matched such that all four axes of the printhead and XYZ stage start and stop virtually simultaneously.
Parameters: E# relative movement of the printhead motor (steps). Positive values should signify printing and negatives values should signify retraction.
X# relative movement of the X axis (steps).
Y# relative movement of the Y axis (steps).
Z# relative movement of the Z axis (steps).
T# exit speed of the movement (steps / s). If this value is unspecified, then then exit speed is zero (complete stop at end of movement).
Return: @MvPrnMtr(#, #, #, #);
There is no return value for exit speed.

Example:
Command: G01 E40 X500 Y500 Z0;
Actions: Move 500 steps in the X direction and 500 steps in the Y direction. The motor-driven printhead dispenses by moving its motor 40 steps across the entire movement.
Return: @MvPrnMtr(40, 500, 500, 0);

G02: Valve Printhead Print With Movement

Command: G02 X# Y# Z# T#;
Actions: Opens the valve on the valve printhead then moves the XYZ stage actuators. The valve will remain open throughout multiple consecutive G02 commands. The valve is closed immediately at the end of movement if not proceeded by another G02 command. Speed and acceleration are matched such that all three axes motors start and stop virtually simultaneously.
Parameters: X# relative movement of the X axis (steps).
Y# relative movement of the Y axis (steps).
Z# relative coordinate movement of the Z axis (steps).
T# exit speed of the movement (steps / s). If this value is unspecified, then then exit speed is zero (complete stop at end of movement).
Return: @MvPrnVlv(#, #, #);
There is no return value for exit speed.

Example:
Message: G02 X30 Y-40 Z0;
Actions: Opens the valve then moves 30 steps in the X direction and 40 steps in the negative Y direction. Closes the valve after movement is complete.
Return: @MvPrnVlv(30, -40, 0);

G11: Motor-driven Printhead Print Without Movement

Command: G11 E#;
Actions: Moves the motor-driven printhead's motor.
Parameters: E# relative movement of the printhead motor (steps). Positive values should signify printing and negatives values should signify retraction.
Return: @PrnMtr(#);

Example:
Command: G11 E-50;
Actions: Retract the motor-driven printhead by 50 steps.
Return: @PrnMtr(-50);

G12: Valve Printhead Print Without Movement

Command: G12 O#;

G12 C;
Actions: Open the valve then close it after a set amount of time.
Pause all operations until a new command specifies to resume.
Close the valve.
Parameters: O/C. Open or close the valve.
O# opens the valve for an amount of time until it closes (microsec). If # = 0 or no value is set, then open the valve until "G12 C;" is messaged.
Return: @PrnVlv(#);
@StpVlv;

Example:
Command: G12 O500;
Actions: Open the valve for 500 microseconds then close it.
Return: @PrnVlv(500);

Example:
Command: G12 O;
G12 C;
Actions: Open the valve until "G12 C;" is received.
Return: @PrnVlv;
@StpVlv;

Special Commands

Ready to Receive

Return: $
Actions: Sent from the microcontroller to the desktop program. This signifies that the microcontroller is ready to receive another command.

Pause Hardware

Command: #
Actions: Pause all hardware operations at the earliest convenience. The current commands will finish execution. Any continuous movements (sequence of movements with exit speeds greater than zero) will also complete before pausing comes into effect. This command is not sent to the microcontroller.

Resume Hardware

Command: %
Actions: Resume all hardware operations if previously paused.

Clear Movement Buffer

Command: &
Actions: Clear all movements from the buffer.
Should only be called if there is an unexpected occurrence (ex. unintentionally hit limit switch) and the buffer needs to be reloaded.

Command Sets

Command Sets are denoted by an asterisk at the beginning of the outgoing command (though this outgoing command is never sent directly to the microcontroller). Command Sets are a series of commands that can be queued up like any other command. Command sets are not executed until all previous serial messages are sent and their respective return messages (@ messages) and complete messages ($) are received. When a command set is executed, the set expands to a series of commands and sent through the serial port or the desktop program will perform an action. Command Sets are meant to be used when command parameters depend on information from previous commands.

Center

Command Set: *Center X# Y#;
Expands To: G00 X# Y#;
Actions: Calculates the midpoint for each axis requested. Outputs a command that moves all requested axes to and the midpoint plus a X and Y offset.
Return: @Mv(#, #, #, #)

Example:
Initial State: X: Position 0, Max Position 1000, Min Position -500
Y: Position 500, Max Position 1000, Min Position 0
Command Set: *Center X100 Y0;
Expands To: G00 X250 Y0;
Actions: Moves axes X by 600 steps and Y by 0 steps. The new positions of each axes will be their respective midpoints (equidistant from their limits). The new parameters for each axis are:
X: Position 350, Max Position 1000, Min Position -500
Y: Position 500, Max Position 1000, Min Position 0
Note that Y did not move because it was already at its midpoint. The X midpoint is at Position 250, and with an offset of 100.
Return: @Mv(250, 0, 0, 0);

Origin

Command Set: *OriginE/X/Y/Z
Expands To: N/A

Actions: All participating axes now use this point as their origin (position = 0) and their max and min range values are recalibrated around this new origin.
Return: N/A

Example:
Initial State: X: Position 0, Max Position 1000, Min Position -500
Y: Position 500, Max Position 1000, Min Position 0
Command Set: *OriginXY
Expands To: N/A
Actions: The new parameters for each axis are:
X: Position 0, Max Position 750, Min Position -750
Y: Position 0, Max Position 500, Min Position -500
Note that the E and Z axes did not take action.
Return: N/A

Set Min Max Positions

Command Set: *SetMinMaxPos EN/M# XN/M# YN/M# ZN/M#
Expands To: N/A
Actions: Sets the current position of axes with the # value. Then sets as either the min (N) or max (M) position values to that # value. The distance between the minimum and maximum positions are maintained. If N or M is not specified, then set the position value only.
Return: N/A

Example:
Initial State: E: Position -500, Max Position 200, Min Position -500
X: Position 0, Max Position 200, Min Position -500
Y: Position 500, Max Position 1000, Min Position 500
Z: Position -250, Max Position 500, Min Position -500
Command Set: *SetMinMaxPos E20 XM1000 YN0 ZN0
Expands To: N/A
Actions: The new parameters for each axis are:
E: Position 20, Max Position 200, Min Position -500
X: Position 1000, Max Position 1000, Min Position 300
Y: Position 0, Max Position 500, Min Position 0
Z: Position 0, Max Position 1000, Min Position 0

Pause

Command Set: *Pause #
Expands To: N/A
Actions: The desktop program will halt serial protocol (outgoing messages to the microcontroller and incoming messages from the microcontroller) will not execute for # milliseconds. Normal procedures will resume after this time. This essentially halts printer operations

for that amount of time.
Return: N/A

Example:
Command Set: *Pause 4000
Expands To: N/A
Actions: Halts printer operations for 4 seconds.

Print Pause

Command Set: *PrintPause
Expands To: N/A
Actions: The desktop program will halt a print sequence until told otherwise via the resume button. During this time, manual commands can be executed.
Return: N/A

Switch Material

Command Set: *SwitchMaterial A/D/AD “(Material Name)”
Expands To:
1. C10 Z command to set the Z actuator to maximum speeds.
2. G00 Z command to retract the Z actuator
3. If “D” is present, then pause the print sequence (pause before deactivating).
4. C10 XYZ commands to set XY actuators and Z actuator to maximum speeds.
5. G00 XY commands to move XY stage to the new printhead position.
6. If “A” is present, then pause the print sequence (pause before activating).
7. G00 Z commands to move the Z actuator to the new printhead position.
8. C10 XYZ commands to set XY actuators and Z actuator to print speeds.
9. C command to set the new printhead.

Actions: Sets new parameters corresponding to the material settings.
Return: Each individual command will elicit a return message as documented in this section of the guide.

Example:
Command Set: *SwitchMaterial AD “Material 1”

Retract Z Actuator

Command Set: *RetractZ
Expands To: G00 Z#
Actions: Moves the Z actuator back to the default position where the default position is 7.5 mm below the upper limit switch.
Returns: @Mv(0, 0, #)

Command Set: *RetractZLimit
Expands To: 1. G00 Z(Large Number)

2. G00 Z#

Actions: Moves the Z actuator until the upper limit then move the Z actuator down 7.5 mm away from the limit switch (default position).

Returns: @Mv, followed by !Lmt, then another @Mv with $ scattered in between.

Statuses

Status messages are denoted by an exclamation point at the start of the incoming message. Status messages are return messages from the microcontroller that are not necessarily preceded by a command.

Limit Switch

Return: !LmtE/X/Y/Z(#,#,#,#);

Actions: During movement actions (G00, G01, G02, etc.), the hardware may run into a limit switch. If it does, then it returns the !Lmt status and stops the movement. This return message details the number of steps taken before hitting the limit switch. The character value(s) following "Lmt" represent which limit switches were hit during movement. numeric value is number of X steps taken, the second is the number of Y steps taken, the third is the number of Z steps taken, and the fourth is the number of E steps taken.

Example:

Return: !LmtEX(1121, 523, 112, 0);

Actions: This movement has completed 1121 X steps, 523 Y steps, 112 Z steps, and 0 E steps before encountering both the E and X limit switches and stopping movement.

Errors

Error messages are returned when incorrect messages are sent or actions out of order are executed. Each error message will be displayed in the serial communications sent and received messages lists and will trigger an error from the error menu.

The first character of an error message is "^" to signify that it is an error message.

The second part of the error message is the type of error that has been triggered.
Syn: syntax invalid (ex. G11 X100, because X is not a command of G11)
Uns: hardware unset (ex. Calling G11 before setting C11)
Cyc: The number of steps per second is exceeding what the microcontroller can handle. Stepper operations will be disturbed.
Ext: Exit speed value is incorrect for a movement. The microcontroller and desktop software are out of sync.

ModiPrint Commands Example

In this example, these messages are sent and received chronologically and assumes no messages were sent before them.

Message	Action	Return	Comment
C00 Connected;	Check connection	Connected	
C10 X T40 D41 P20 L42 S500 A100;	Sets parameters for the X Axis.	SetXAxs(40, 41, 20, 42, 500, 100)	
C10 Y T40 D41 P20 L42 S500 A100;	Sets parameters for the Y Axis.	SetXAxs(40, 41, 20, 42, 500, 100)	The Y Axis has overlapping GPIO Pins with the X Axis. This software does not handle errors for overlapping GPIO Pins. Strange behavior may occur.
C10 Y T45 D46 P20 L47 S500 A100;	Sets parameters for the Y Axis.	SetYAxs(45, 46, 20, 47, 500, 100)	Overwrites the previous C10 command. This sequence is no longer erroneous.
G11 E50;	Nothing	Error	No motor-driven printhead was set, so this command is invalid.
C11 T20 D21 P20 L70 S5000 A1000; C10 Z T22 D23 P20 L70 S500 A100;	Sets parameters for a motor-driven printhead and its associated Z Axis.	SetMtrPh(20, 21, 20, 70, 5000, 1000) SetZAxs(22, 23, 20, 70, 500, 100)	
G11 E50;	Prints from the motor-driven printhead.	PrnMtr(50)	
G12 O500;	Nothing	Error	No valve-based printhead was set, so this command is invalid.
G01 E40 X30 Y-40 Z0;	Prints with the valve printhead while moving along a 30 x 40 diagonal.	MvPrnVlv(30, -40, 0)	

Supported G-code

This section contains the g-code that is supported when reading and processing uploaded .gcode files. If a .gcode file contains lines that are not supported by ModiPrint, then those lines will simply be ignored during the conversion process. See here for a full list of all RepRap flavor g-code. To be clear, ModiPrint only reads RepRap flavor g-code, but not all of RepRap g-code is supported. Support g-code includes:

; Any characters within the same line and following a semicolon is ignored by ModiPrint. These extraneous characters are meant to be comments that are annotated and read by users. Any characters within the same line as and preceding a semicolon is read and processed by ModiPrint.

G00 & G01: G00 and G01 commands do not have a distinction in ModiPrint. They represent XYZ movement or printing. If positive E movement is present, then ModiPrint will convert this g-code line to a print command. Otherwise, it is movement without printing.

GX: This is not a conventionally used g-code command and will not be appear in slicing software or wikis. This feature was implemented to make manually writing tool paths an easier task. GX is a command that converts to a print command without reading E movement. For an example of how GX is implemented, see "15mm Grid 500µm Spacing" in the "Tool Path Samples" folder.

T: T signify switching printheads. ModiPrint will switch to a printhead corresponding to the material with the same G-code ID parameter. The T g-code line will be converted to a set of ModiPrint commands that retract the current printhead, activates the new printhead, then maneuvers the new printhead to the same position as the old.

G90 & G91: G90 and G91 sets absolute and relative respectively for reading X, Y, and Z values of G00, G01, and GX. For example, G90 followed by G00 X0 will move the XYZ stage to the X origin. G91 followed by G00 X0 will yield no movement.

G92: G92 sets the current position to a new absolute coordinate for X, Y, Z, and E. For example, G92 X0 Y0 E0 will set the current location as the new origin for all except the Z actuator. All proceeding G00, G01, and GX g-code lines will be affected if the system is reading absolute coordinates (G91).

M82 & M83: M82 and M83 sets absolute and relative respectively for reading E values of G00 and G01.

RepRap G-code Example

The following example details how ModiPrint converts a g-code file. This is an excerpt taken from the beginning of the g-code file that was generated from concentric cylinder example in the software operations section.

```
; generated by Slic3r 1.3.0 on 2019-09-07 at 19:03:54

; external perimeters extrusion width = 0.12mm (0.30mm^3/s)
; perimeters extrusion width = 0.12mm (0.57mm^3/s)
; infill extrusion width = 0.12mm (0.76mm^3/s)
; solid infill extrusion width = 0.12mm (0.19mm^3/s)
; top infill extrusion width = 0.12mm (0.14mm^3/s)

; external perimeters extrusion width = 0.46mm (1.31mm^3/s)
; perimeters extrusion width = 0.58mm (3.36mm^3/s)
; infill extrusion width = 0.52mm (3.98mm^3/s)
; solid infill extrusion width = 0.58mm (1.12mm^3/s)
; top infill extrusion width = 0.58mm (0.84mm^3/s)
```

Comments.
These are irrelevant to the operation of ModiPrint.

```
M107
M104 S205 T0 ; set temperature
M104 S205 T1 ; set temperature
G28 ; home all axes
G1 Z5 F5000 ; lift nozzle

; Filament gcode

; Filament gcode

M109 S205 T0 ; set temperature and wait for it to be reached
M109 S205 T1 ; set temperature and wait for it to be reached
G21 ; set units to millimeters
```

Comments and g-code that is not supported by ModiPrint. These lines are irrelevant to the operation of ModiPrint.

```
G90 ; use absolute coordinates
M82 ; use absolute distances for extrusion
```

Interpret the following in absolute coordinates.

```
G92 E0
T0
G92 E0
```

Switch to the material corresponding to T0.
The absolute position of E is set to 0.

G1 Z0.100 F7800.000	Move the Z actuator up with no printing.
G1 E-2.00000 F2400.00000	E retraction and F settings are not supported and is thus ignored.
G92 E0	The absolute position of E is set to 0.
G1 X-2.674 Y-3.038 F7800.000	Movement without printing.
G1 E2.00000 F2400.00000	Printing without movement is not supported g-code.
G1 F4214.25	F Setting is not supported and is thus ignored.

```
G1 X-2.122 Y-3.447 E2.00092
G1 X-1.509 Y-3.756 E2.00185
G1 X-0.853 Y-3.957 E2.00277
G1 X-0.172 Y-4.044 E2.00369
G1 X0.514 Y-4.015 E2.00461
G1 X1.185 Y-3.870 E2.00554
```

Movement with printing. Since the E value is incrementing positively on every line, ModiPrint reads these lines as print commands.

Hardware Operation

Dispensing Components

This section is about the selection and use of the components that interface with the bioink. This guide recommends syringe barrels with variable pistons and nozzles. While many manufacturers provide these systems, this guide will specifically refer to the catalog of Nordson EFD. This guide only covers the essentials of dispensing components and it is recommended that the user explore other options of this very customizable system.

Pneumatic Extruder: Choosing Dispensing Components

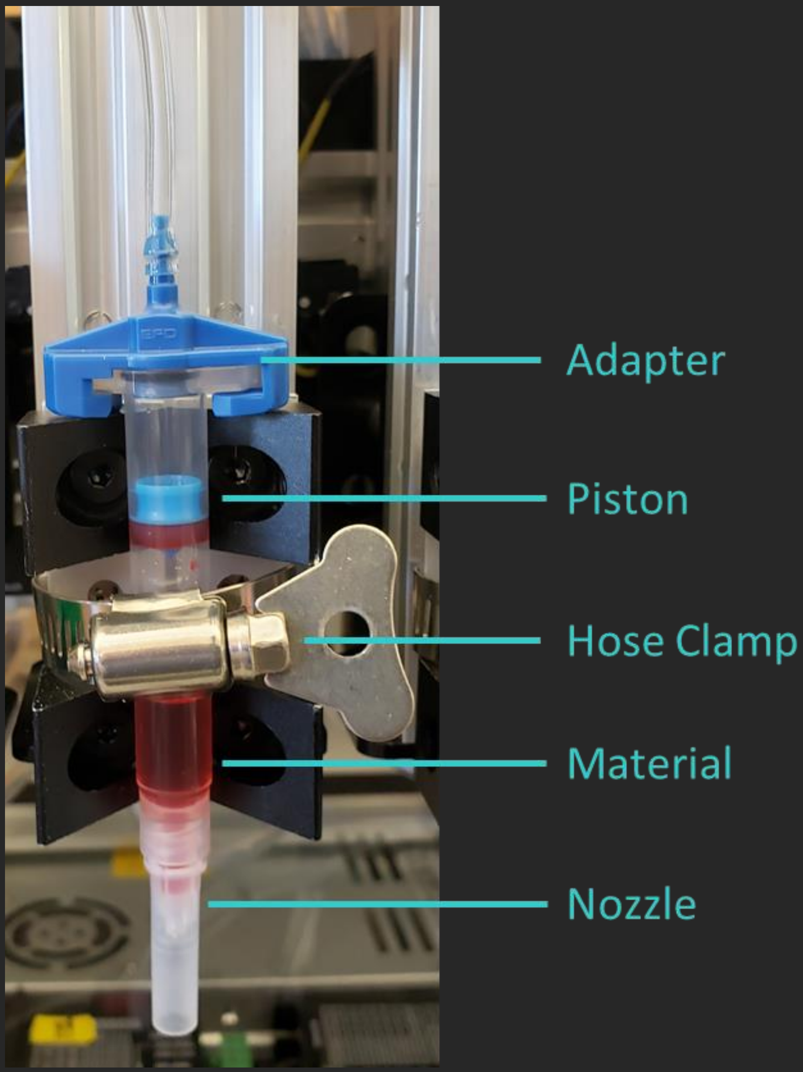
Syringe Barrel: The syringe barrel acts as the material reservoir. The only factor to consider is how much material each print will consume. A 3 mL syringe barrel is typically enough for most prints and is the defaulted in the parts list and Sketchup designs. A larger syringe barrel will require an alternative build of the syringe barrel mount.

Adapter: The adapter connects the syringe barrel to the pneumatics and is fit to specific sizes of the syringe barrels. Therefore, buy a 3 mL adapter to fit 3 mL syringe barrels. A larger syringe barrel will require an alternative build of the syringe barrel mount. The hose trap is an optional purchase, but it is recommended for the equipment's safety for use with the vacuum feature in ModiPrint's pneumatic extruders.

Piston: The piston fits into the syringe barrel and is used to evenly distribute the force of the gas pressure and reduce material waste. The Blue LV Barrier piston is the only piston that is compatible with the vacuum feature in ModiPrint pneumatic extruders. If material viscosity is high enough to not require the vacuum feature, then the White piston can be used. Highly viscous or materials with strange properties may require the other pistons to dispense optimally.

Nozzle: The nozzle is Luer lock fit to the bottom of the syringe barrel. The most important property of the nozzle is the inner diameter. High resolution prints should use a finer nozzles such as this 100 µm inner diameter nozzle which comes in chamfered and general purpose variants. More clog-prone, highly viscous materials or materials with difficult-to-print properties may necessitate larger inner diameter tips with specialized properties (like PTFE-lined).

If printing into FRESH support baths, a high outer diameter tip disrupts the self-healing nature of the gelatin.



Microdispensing Inkjet: Choosing Dispensing Components

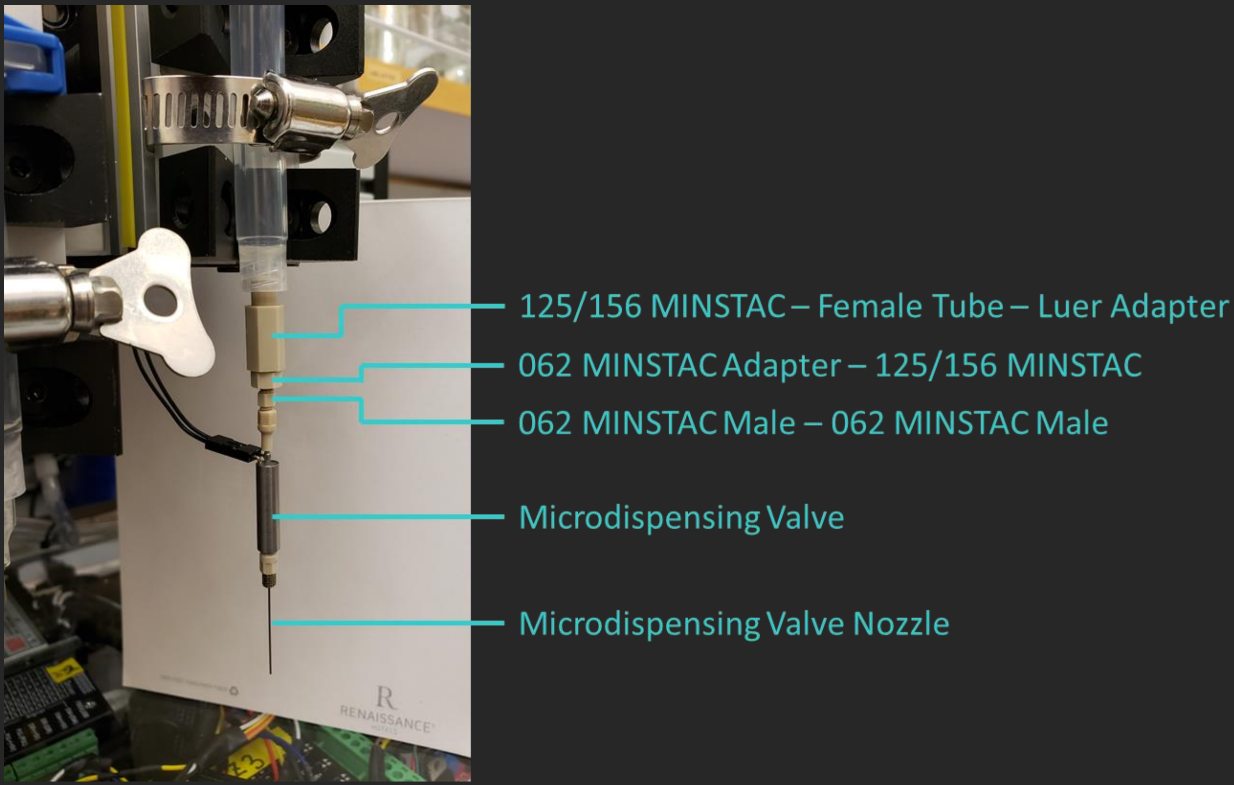
Syringe Barrel: The syringe barrel acts as the material reservoir. The only factor to consider is how much material each prints will consume. A 3 mL syringe barrel is typically enough for most prints and is the defaulted in the parts list and Sketchup designs. A larger syringe barrel will require an alternative build of the printheads.

Adapter: The adapter connects the syringe barrel to the pneumatics and is fit to specific sizes of the syringe barrels. Therefore, buy a 3 mL adapter to fit 3 mL syringe barrels. A larger syringe barrel will require an alternative build of the syringe barrel mount.

Piston: The piston fits into the syringe barrel and is used to evenly distribute the force of the gas pressure and reduce material waste. Unlike the pneumatic extruder, Blue LV Pistons do not apply to the microdispensing inkjet because a vacuum feature is present for this printhead. As the microdispensing inkjet is intended for low viscosity materials, a White Piston is recommended.

Solenoid Valve: For Luer lock compatibility and variable nozzle sizes, use Minstac Inlet / Minstac Outlet VHS valves. Besides that, the differences between each valve is minute. INKX0514300A seems suitable for most materials and the supporting components in this guide complement its voltages. Deviation to another valve will require deviation from the default supporting electronics (24V power supply, LM2596 Converter) in the parts list.

Nozzle: The nozzle needs to be Minstac compatible to interface with the suggested VHS solenoid valve. It is best to have smaller inner diameters for lower volume droplet dispensing. For printing in FRESH gelatin slushie, longer nozzle lengths enable a bigger Z range and smaller outer diameters allow better self-healing of the support gel. 0.5 mm outer diameter is an appropriate nozzle size for FRESH. If the material has difficult-to-print properties like stickiness, high viscosity, etc. consult The Lee Co. engineers.



Loading Bioink

1. Attach the nozzle to the Luer lock of the syringe barrel.
    a. This, as the first step, prevents material from leaking out of the syringe barrel in the upcoming steps.
    b. If the material is prone to clogging the nozzle, attach a tip cap instead.
2. Fill the syringe barrel with material.
    a. Be sure to leave some space for the next step.
3. Insert a piston into the syringe barrel. The specific type of piston may come with special loading instructions.

   a. White pistons and most other pistons need to be pushed down until the piston contacts the material.
   b. Blue LV pistons need a gap of air between it and the material.
4. Attach the loaded syringe barrel to the adapter.
5. Insert the syringe barrel to the syringe barrel mount on the printhead and tighten the hose clamp.
   a. This step may affect printhead offsets which are relevant in multi-printhead operations. For consistent positioning and accurate printhead offsets, position the syringe barrel and tighten the hose clamp consistently every time.
6. Dispense with the printhead to remove air in the nozzle.
   a. This is done within the ModiPrint desktop program by setting the printhead and pressing the valve open button.
   b. If the material is prone to clogging, be prepared to begin printing immediately after this step.

Pneumatic Regulators

The pneumatics regulators in ModiPrint are operated by hand. The very small regulators recommended in this guide have tiny pressure gauges that do not accurately represent the low pressures required in bioprinting. Therefore, a digital pressure regulator is recommended for setting accurate pressures.

Pressure Regulator

The gauge on the pressure regulator does not accurately represent the low pressures required of bioprinting. To circumvent this, the hardware section of this guide recommends incorporating a detachable electronic pressure gauge downstream of the pressure regulator.

1. Connect the digital pressure gauge to the pressure line.
2. Turn on the pressure source.
3. Adjust the pressure regulator knob until the digital pressure gauge reads the correct value.
   a. The pressure regulator has some delay during pressure changes. Wait several seconds after changing the pressure to confirm that the pressure is stabilized at the correct value.

Vacuum Regulator

The purpose of attaching a vacuum to the pneumatic extruder is to prevent leakage of low viscosity materials. When closed, the solenoid valve of the pneumatic extruder connects the syringe barrel to the vacuum line, flushing out residual pressure and counteracting gravity's tendency to drag material out of the nozzle. This feature would not be necessary for a higher viscosity material that does not leak.

Only a low amount of vacuum force is required for most low viscosity materials (typically between 0 and -1 psi). Vacuum forces of that magnitude are difficult to measure, so unlike the pressure regulator, there will be no digital gauge to support this operation. Fortunately, the vacuum regulator only needs to be set once and should support a variety of print scenarios and low viscosity materials for all printheads.

1. Turn on the pressure and vacuum source.
2. Set the pressure regulator to the intended printing pressure.
3. Tighten the vacuum regulator such that it is shut.
    a. This is a precautionary step to ensure that material is not immediately drawn into the trap.
4. Load the material into the syringe barrel with a Blue LV piston. Attach the loaded barrel to the barrel adapter.
5. Open the valve on the pneumatic extruder. Close it immediately after material has dispensed and wipe off remaining material from the nozzle.
    a. If the material requires the vacuum feature, it should leak from the nozzle after pressure cutoff. If it is not leaking, then the material does not require this vacuum feature.
6. Loosen (increase vacuum) the vacuum regulator until material has stopped leaking.
7. Repeat steps 5-6 until the material immediately stops dispensing with the closure of the valve.
8. With the valve closed (and thus the syringe barrel is connected to the vacuum line), watch the material for 5 minutes. If bubbles begin to form, then tighten (decrease vacuum) the vacuum regulator and repeat steps 5-8.

The vacuum feature on the pneumatic extruder is similar to the dispensers of Nordson EFD. Their manual (page 16) may yield more insight.

Sterile Prints

ModiPrint was designed to be compact enough to fit in the sterile environment of a laminar flow hood. This system was also designed to fit autoclavable dispensing and pneumatic components.

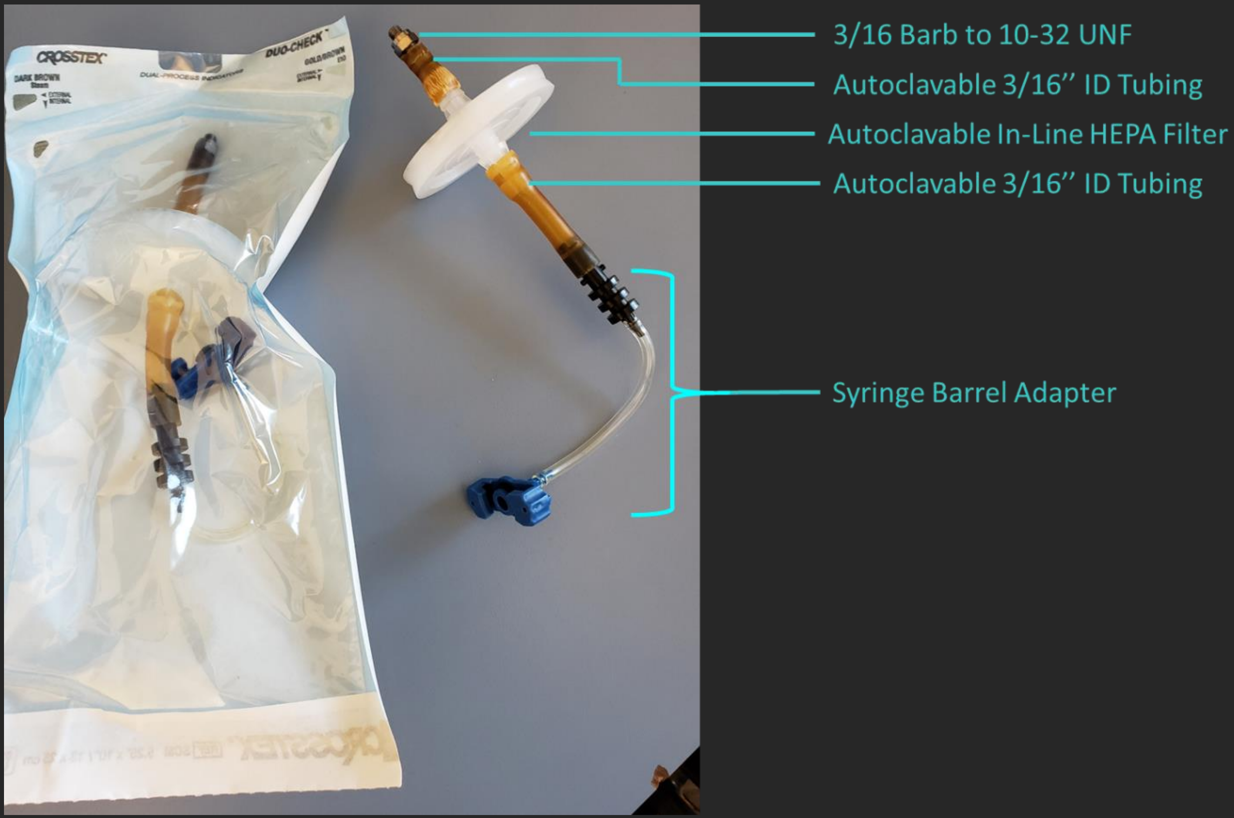
Autoclaving Preparations

Before the print, autoclaved components and sterile, pH buffered inks must be prepared. How the inks will be sterilized will vary, but common methods include long exposure to UV (30+ minutes), filter through a 0.22 or 0.45 µm filter, and autoclaving.

Pneumatic Components to Autoclave:

- Autoclavable 3/16'' ID Tubing
- Autoclavable In-Line HEPA Filter
- 3/16 Barb to 10-32 UNF
- 3cc Syringe Barrel Adapter *without* Filter Trap

These components can be pieced into an assembly that screws right onto a pneumatic extruder's solenoid valve. Below is an image of this assembly inside and outside of an autoclave bag.



Dispensing Components to Autoclave:

- Syringe barrel
    - Nordson EFD syringe barrels are autoclavable.
- Syringe barrel piston
    - Nordson EFD pistons are autoclavable
- Nozzle
    - Nordson EFD metal nozzles are autoclavable.
    - Autoclave more nozzles than needed in case of clogging.
    - If the nozzle comes with a plastic cap, do not autoclave the cap as it will melt.

If using the microdispensing valve, do not autoclave the VHS components. Autoclaving these components will cause permanent damage. These components should be flushed with 70% ethanol, then water/buffer to sterilize.

Before the Sterile Print

Before sterile printing, the following must occur:

1. Move the bioprinter into a sterile environment.
2. If possible, expose the machine to UV light. Short term exposure to UV (a few minutes) should not damage any components.
3. Thoroughly sterilize the printer with 70% ethanol with special attention giving to the print surface and printheads.
    a. Do not expose the electronics to the ethanol.
4. Attach the autoclaved pneumatic components to the appropriate printheads.
5. Load the autoclaved dispensing components with the bioink within the sterile environment.

Unclogging Nozzles

Unclogging the Pneumatic Extruder

To prevent pneumatic extruder nozzle clogs:

- Do not load the material until right before printing. At the very least, do not push the material into the nozzle and idle the printhead. Idling a printhead with a nozzle full of material may cause the small volumes of material to dry and clog.
- Frequent clogging may be a sign of a nozzle with too small of an inner diameter or a material that is too concentrated.
- If idling while the nozzle is full of material is necessary, consider dispensing a droplet small enough that it remains attached to the nozzle opening. This will prevent drying and clogging for at least a few minutes (pictured below).

To unclog a pneumatic extruder nozzle:

- Increase the pressure and attempt to dispense again.
- While attempting to dispense, jiggle the nozzle gently by hand.
- While attempting to dispense, hold a beaker of hot water up to the nozzle.
    - Do not attempt this with the microdispensing inkjet. The microdispensing valve is very sensitive to temperature.

Unclogging the Microdispensing Inkjet

A major cause of the VHS valve clogging is the passage of particles through valve. As such, to prevent microdispensing inkjet clogs:

- Ensure your solution is free of particles. A filter should be used to prevent particles from incoming gas. A safety screen can be fitted in between the VHS valve and its adapter as a last line of defense (The Lee Co. #INMX0350000A).
- The valve should be flushed with deionized water or ethanol after use.

To unclog a microdispensing inkjet:

- Repeatedly pulse the valve at 500 Hz under pressure. This is not guaranteed to purge the valve and ideally, a clogged valve situation should be avoided.
    - The "500Hz Unclogging.mdpt" file in the "Tool Path Samples" folder can be loaded and executed as any print sequence. It will pulse the valve at 500 Hz for 500 cycles. Be sure to set the microdispensing inkjet under the "Control" menu before executing this sequence.

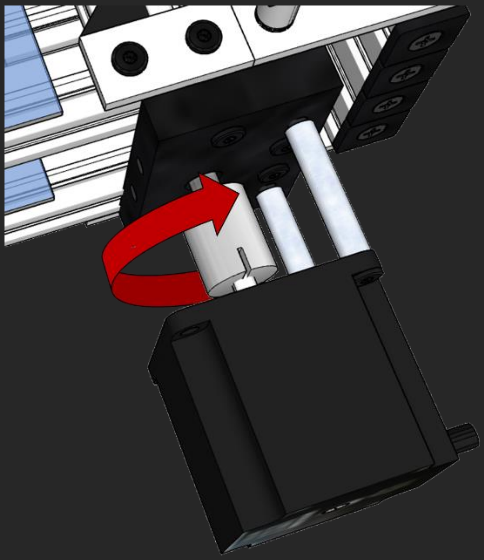
Maintenance

It is recommended to check the following every few months:

Y Actuator Alignment

The two Y actuators are ideally at the same position along the Y axis. If they are not, then the X actuator will not be parallel with the X axis.

To align the Y actuators, manually move the actuators by rotating the motor couplings while power sources are off. If the limit switches of both Y actuators are in the same position along the Y axis, then simply use the limit switches as guides to maneuver the Y actuators to the same position. Otherwise, a measurement tool can be used to determine the positions of the Y actuators. While maneuvering the actuators, ensure that both motors are rotated at the same speed.



Lock Collar Position

Lock collars couple the actuator's acme screw movements with the actuator's load. There are two lock collars for every actuator, one on each end of the acme screw. If they are off position, movement of the acme screws can result in no movement of the load. Jiggle the acme screws along the direction of the actuator's movement. If the screw is loose, then the lock collar needs to be readjusted.

To readjust the lock collar, unscrew the screw that holds the collar in place. Ensure that the lock collar on the opposite end is pressed against the mounting plate by pushing to the acme screw. Press the loose lock collar against its respective mounting plate and secure it back to the acme screw. Jiggle the acme screw again to ensure that the actuator is coupled correctly.

Example Protocols

FRESH Support Bath

FRESH stands for freeform reversible embedding of suspended hydrogels. It is a temporary support that suspends liquids in 3D space as they crosslink. The FRESH support bath can be suspended with the crosslinker itself to enable crosslinking on print. It is inexpensive, compatible with a variety of materials, and enables high resolution 3D printing.

See here for the original FRESH paper and here for an alternative FRESH protocol with an accompanying video.

Alginate

Alginate is an inexpensive and very easy to use material with the FRESH system. It is at an ideal viscosity for controlled material deposition and easy to prepare. Normal alginate is no functionalized with RGD binding sites and is typically used with other hydrogels to support live cells.

Alginate Preparation:

1. Dissolve 2% wt/v alginate with DiH2O at 65°C stirred.
    a. Expect this process that take several hours.
    b. For live cell prints, dissolve the alginate in a buffer such as Earle's Salts with 10 mM HEPES instead of DiH2O.
    c. For sterility, the alginate solution can be passed through a 0.22 µm filter. Use larger surface area filters as this process is prone to clogging.
2. This solution can be stored for several months at 4C or room temperature.
3. A small drop of food coloring can be added at any time after preparation. This will help visualize the otherwise transparent alginate during the printing process.

Gelatin Puck Preparation:

1. Mix 11 mM CaCl2 with DiH2O. A 1L bottle should contain 1.22078g of CaCl2.
    a. Shaking the bottle for 30 seconds should be enough to homogenize the calcium.
    b. For live cell prints, it is recommended to use a buffer such as Earle's Salts with 10 mM HEPES instead of DiH2O. Phosphate will form calcium phosphate with the CaCl2, so do not use phosphate-based solutions (such as PBS) to make the gelatin solution.
    c. Calcium is used here to crosslink alginate. If other inks are used, other crosslinkers can be added at this step. For example, if the ink is fibrinogen, thrombin can be added here instead of CaCl2. If an ink does not require a crosslinker (like collagen), no additive is needed.
2. Dissolve 4.5% wt/v gelatin with 150 mL of the solution made in step at 65°C stirred.
    a. Expect this process that take several hours.
    b. For sterility after dissolving, this solution can be passed through a 0.22 µm filter. Warm gelatin will pass through the filter with ease whereas room temperature gelatin is viscous or solid. So, filter immediately after dissolving.
3. Store the gelatin/calcium solution in a 500 mL mason jar at 4C for 12 hours. The solution should be fully gelled after this time.
    a. Storage at this step is possible for up to one week at 4C. Any longer and the gel solution will start bleeding liquid and degrade in physical properties. For storage more than 12 hours, it is

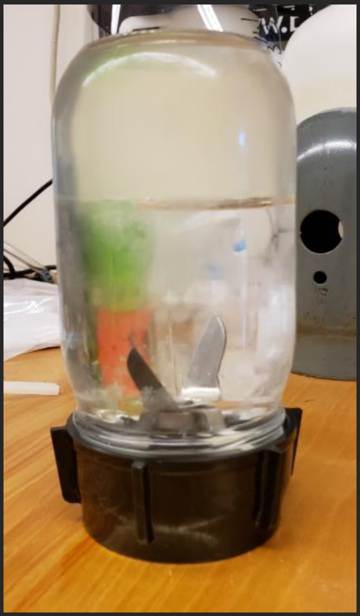
recommended to keep the gelatin puck on ice at 4C. Best practice is to process the puck (next step) as soon as possible.

Gelatin Slushie Preparation:

1. Add ~100 mL (doesn't have to be exact) of 4°C 11 mM CaCl2 solution to the mason jar. Use a spatula to separate the gelatin puck from the mason jar walls.
    a. If this step is performed improperly, a large amount of gelatin will remain stuck to the jar walls after the blending step.
    b. Ideally, the gelatin puck should be completely dislodged at all sides without breaking the puck.
    c. We prefer a cell scraper for this step.
2. Fill the rest of the jar to the brim with 4°C 11 mM CaCl2.
    a. Ensure there is as little empty space as possible by filling the jar to the brim. Expect to spill in the next step.

Controlling the temperature at and past this step is paramount to producing high quality FRESH slushie. Make sure to cool the material with consistent timing and temperature. Avoid warming the material with body heat. Keep the material on ice or in the fridge whenever possible.

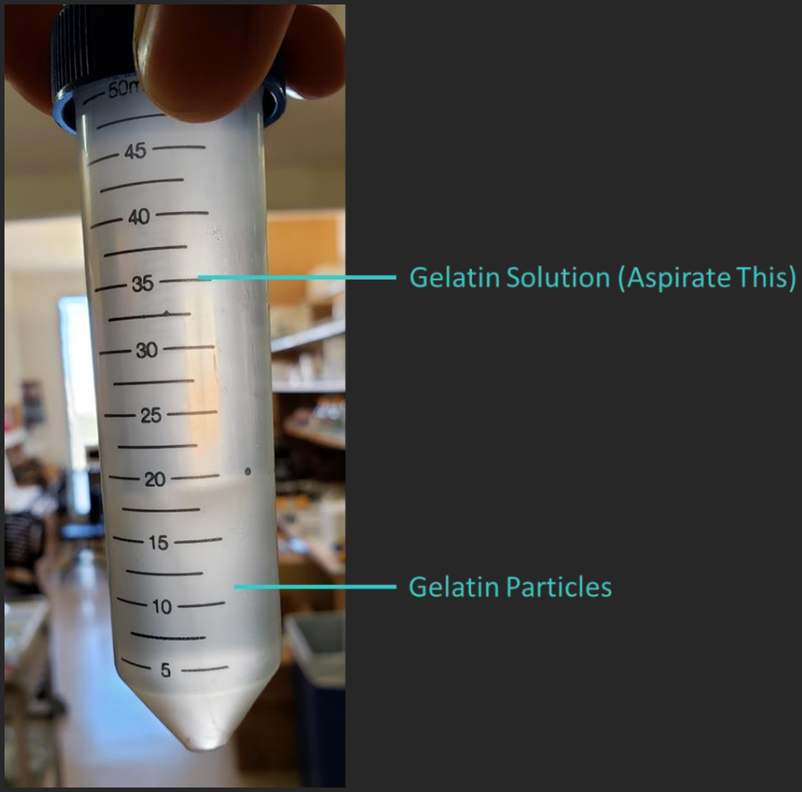
3. Swap the lid of the mason jar with the blender blade assembly. The order of parts should be: Jar -> rubber O-ring -> blender blade -> the plastic part that interfaces the blender blade with the motor.
    a. For sterility, the blender blade and O-ring can be autoclaved. Do not autoclave the blender housing as the plastic housing will deform under autoclave conditions. Although the plastic housing does not come in contact with the gelatin, it is recommended that is be sterilized via UV exposure and/or ethanol for sterile use.



4. Chill the mason jar at -20°C in the freezer until ice has just started forming.
    a. This step will allow the gelatin to maintain its mechanical integrity during blending.
    b. This step should take as long as 2 hours if all materials were at 4°C before the freezing process.
    c. Wipe off condensation from the jar before freezing so that the surface of the jar does not cool faster than the interior.

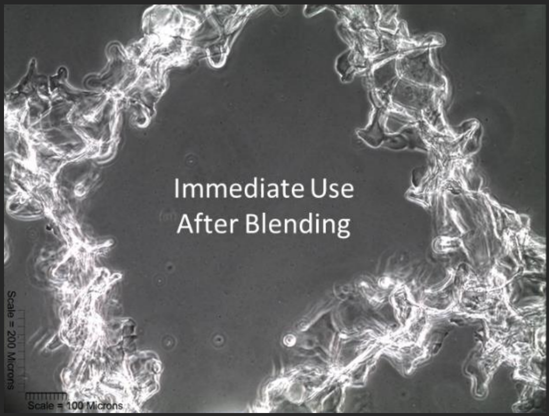
   d. Leaving the jar in the freezer for too long may cause the jar to burst from excess ice formation. Chilling the jar for too short of a time may cause the gelatin to disintegrate during the blending step. Check the jar every 5 minutes and begin the next step only after ice formation.

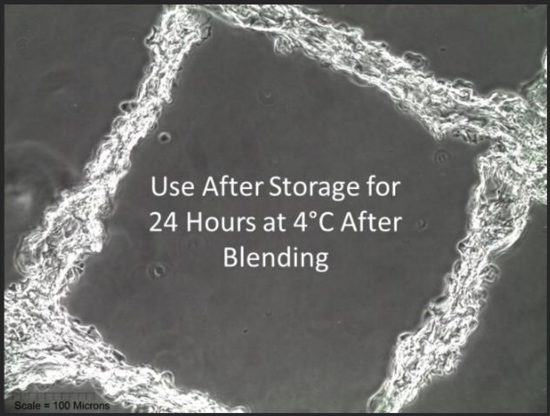
5. Cool the centrifuge to 0°C a few minutes before the next step.
6. Blend on pulse speed for 2 minutes.
   a. If successfully dislodged in step one, the gelatin puck will peel away from the container during blending. This process can be felt within the first seconds of the blending process.
   b. The originators of FRESH and use an Osterizer Beehive blender. For best results with this protocol, use that specific model too.
7. Transfer the contents to a 50 mL conical tubes via pipettor.
8. Centrifuge the blended contents at 3500G and 0°C for 4 minutes.
   a. If the centrifuge cannot handle such high Gs, then the spin time can be increased.
9. Within the conical tubes, a solid layer of gelatin particles may be visible along with a layer of gelatin solution above it and a thin layer of white foam at the very top. Aspirate all but the bottom layer of gelatin particles.
   a. The top layers should be behave as low viscosity liquids and the particle layer should behave as a solid or very viscous liquid. If the entirety of the material behaves viscously, then this indicates that the gelatin has melted. To prevent melting in future steps, chill the material for longer durations in step 4 and decrease exposure to heat throughout the entire process.



10. Resuspend the gelatin particles in 11 mM $CaCl_2$ solution and vortex thoroughly.
   a. If properly dispersed, the gelatin will behave as a liquid. Invert the conical tube to see that the particles are dispersed properly.

11. Repeat steps 8-10 at least 2 times. The foam raft at the very top should disappear by the end of this process.
12. Chill the support bath in the fridge for 24 hours before use.
    a. This step reduces the variance of filament diameters (see below).





13. Best practice would be to use the support bath close to this 24 hour timing. Stored at 4 Celsius, the bath will quickly degrade along with print quality.

Gelatin Slushie Plating:

1. Move the mass of gelatin particles down to a petri dish.
    a. Tissue culture plastic is not necessary.
    b. The gelatin particles should come out as mostly rigid shape of the conical tube. As such, the gelatin particle structure can be flicked out of the conical tube as one continuous blob. This has much less waste than scooping the particles out and it demonstrates the mechanical integrity of the particles.
    c. If the particles are mushy, then a previous process was performed incorrectly. Skipping Step 12-13 of the gelatin slushie preparation can cause this. The best quality FRESH gels come out in the shape of the conical tube as shown below.

2. Plate the gelatin particles to petri dishes.

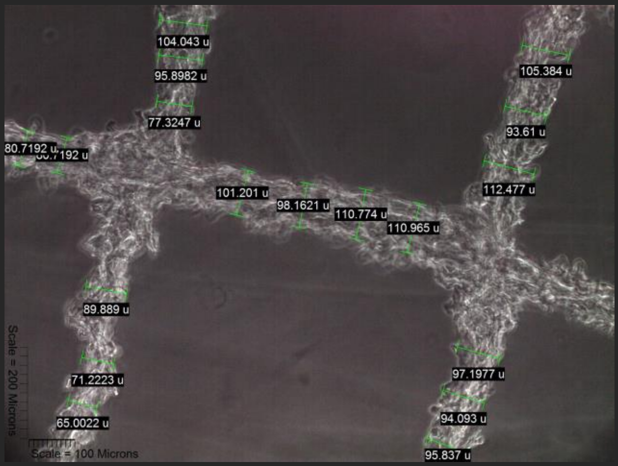
   a. Approximately 5 mL of the slushie can fill a 35 mm petri dish.
3. Centrifuge the dish down at 1500 G for 5 minutes to remove bubbles and flatten the structure.
   a. Higher Gs may break the dish.
   b. The dishes may need to be rotates and spun again to evenly distribute the particles.
4. Print into the dish while it is still cold. Alternatively, store the dish at 4C temporarily.
   a. Extended storage may cause the particles to melt. It's not advised to store the petri dishes at this step for more than 2 days else print quality will degrade.

Example Prints & Optimization

Filament Quality

Printing grid shapes is a good way to learn about the machine's operation, learn about the physical properties of the material, check the quality of the print, and optimize material filament diameter. An example g-code file is labeled as "15mm Grid" and can be found under the "Tool Path Samples" folder. The example print begins at the top left corner, so leave 15 mm of runway in the positive X and negative Y directions.

Hereafter, spacing will refer to the distance between each line. Smaller spacing improve the structural integrity of the print but makes it difficult to see the quality of individual filaments. The inverse is true for larger spacings. A variety of spacing sizes is provided in the folder for this optimization process. Below is a phase contrast images of 100 µm 2% alginate filaments printed in a 500 mm spacing grid.



Actual prints would typically have filaments running in parallel (assuming 100% infill) and individual filaments would ideally fuse together, so no overlap or individually viewable filaments would occur. However, determining filament diameter is still important for setting the XY spacing parameter during g-code processing and viewing filament quality is useful for debugging. Selecting an appropriate spacing in this step and properly optimizing filament diameter is important for determining parameters for future prints.

Filaments can be viewed under a light microscope or, for better image quality, a phase contrast microscope to quickly gauge the quality of the print. Things of note:

- Decreasing filament diameter can be achieved by decreasing pressure, increasing print speed, and decreasing nozzle inner diameter. The inverse is true for increasing filament diameter.
- Food coloring can be added to the material before printing. This allows visibility to an otherwise transparent material.
- For pneumatic extruders, materials need to be mildly viscous to print in decent resolutions. Aqueous inks (such as fibrinogen) need to be mixed with more viscous materials (such as alginate)

else the dispense rate of the low viscosity material will be uncontrollable. Low viscosity materials are better suited for motor-driven dispensers which can directly control dispensing rate.

Complex Shapes

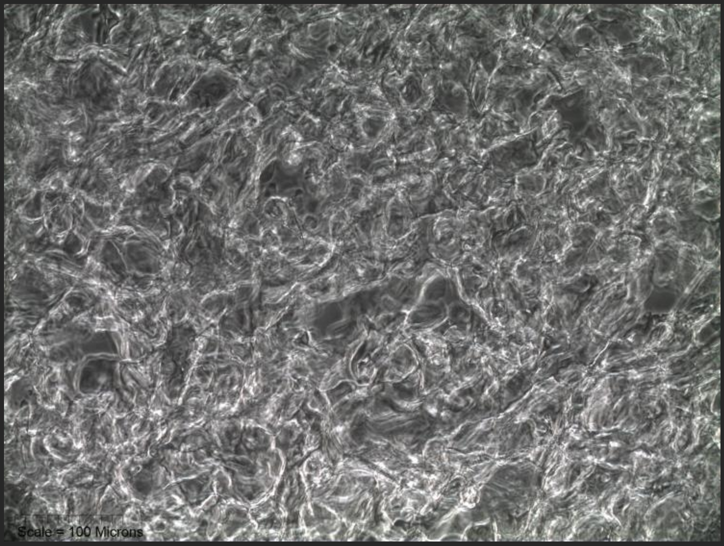
Quantify filament diameters before performing this test.

Circles are one of the more challenging tasks for 3D printing. Since there is no such thing as true arc movement, slicing software and controllers interpolate an arc into many line segments. As such, executing a circular tool path requires fast, sequential actions from both software and hardware components. Transitioning between line segments also causes deceleration of the XYZ stage and the consequent deposition of excess material from pneumatically driven dispensers. As such, circles are also a good test of how well this machine is configured for fast cornering with acceleration and junction deviation settings.

Included with the user guide in the “Tool Path Samples” folder is a g-code file of a 16 mm diameter disc with 150 µm XY spacing. When viewed on NCViewer, it is apparent that the print begins at the center of the disc and requires 8 mm of space in the X and Y direction. Notice that line segments are shorter near the center of the disc where the circle radius is smallest. Expect the printer to be more stressed when printing these tighter arcs and for print quality to vary between the inner and outer sections of the disc.

Unless the expected filament diameter is 150 µm, do not use this file to optimize the print. Users must generate custom geometries with XY spacing that is equivalent to the expected filament diameter.

If filaments are properly spaced apart, then they should fuse together into a dense sheet where individual filaments are indistinguishable (see the image of printed 2% alginate below). If filaments are printed too close together (XY spacing is too small) then excess material will bleed from the intended geometry. If filaments are printed too far apart, then individual filaments will be clearly distinguishable from each other and the structural integrity of the print will be compromised. If printing with FRESH, it is recommended to dye the material (food coloring works well) in addition to using microscopy to see such irregularities.



It is encouraged that users also test complex geometries that are sliced with rectilinear fill patterns. Included with the documentation in the "Tool Path Samples" folder is a g-code file of a 25 mm femur with 200 µm XYZ spacing and its accompanying OBJ file.

Multi-Material Prints

Multi-material prints add additional complications to the process. There are two issues that can immediately be seen from a multi-material test print:

Nozzles clogging when printheads are idle: The continuous flow of material in a single-material print would prevent this from occurring. But when a nozzle is idle, the small volumes of material within it can dry from exposure to air, or clog from previous exposure to crosslinkers. Testing multi-material prints is a good way to see if prints require additional assistance to unclog.

If nozzles are clogging during multi-material prints, consider setting the "Pause after activating/before deactivating" toggle to true under material settings. This feature will stop the print immediately before or after switching printheads. While paused, manual control will be enabled to unclog the nozzle before resuming print.

The "Tool Path Samples" folder contains the 2 material 6 mm diameter concentric cylinders that served as the example in the "Generating G-code" section of the guide which is suitable for a multi-material print test.

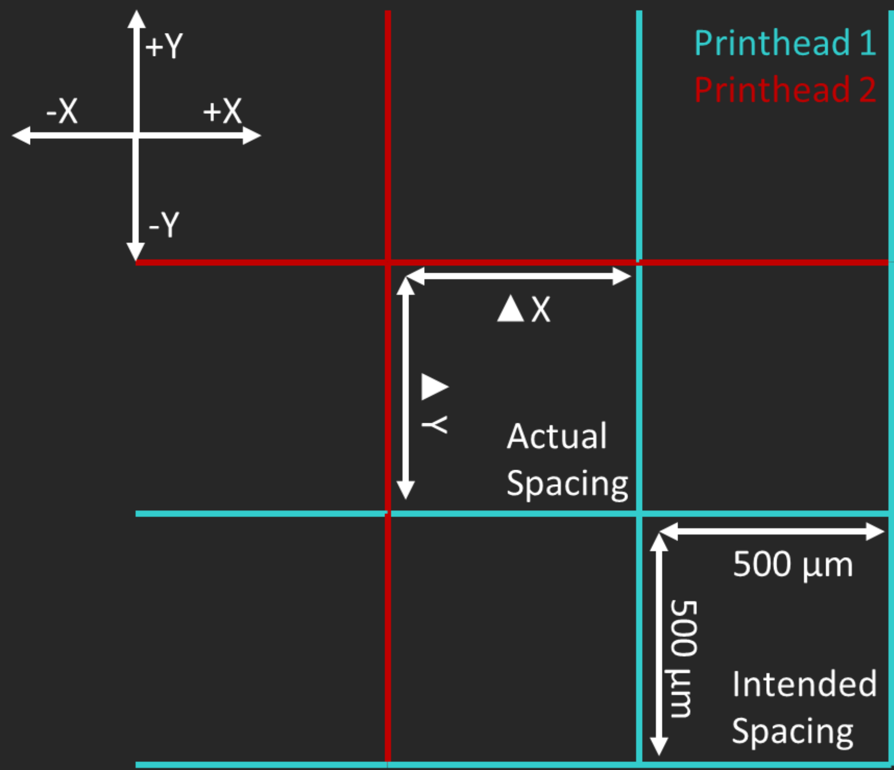
Improperly calibrated printhead offsets: The basic calibration process involves positioning printheads with the naked eye. This process can position printheads quite precisely with some careful, methodical operation. However, if for more precisely calibrated printhead offsets, print patterns and deviations can be measured via microscopy.

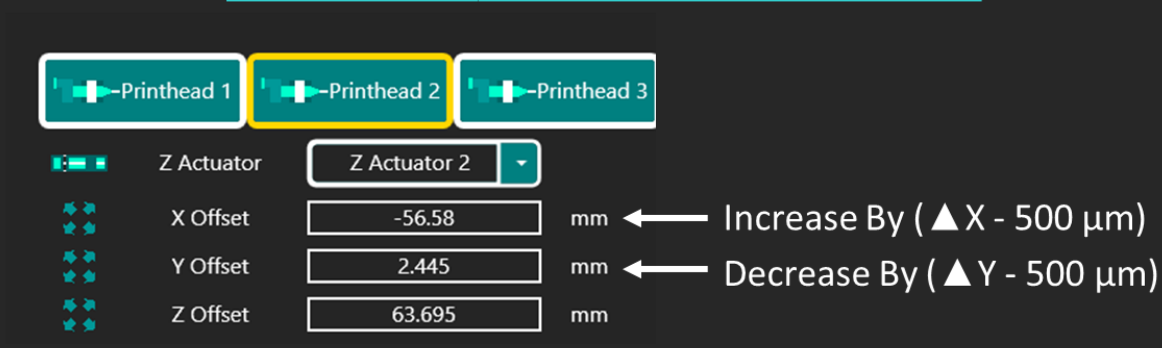
Included in the "Tool Path Samples" folder are two files for multi-material grids. These 15 mm grids are generated with two different printheads alternating the dispensing of each line. These multi-material grids come in 500 µm and 1 mm spacing variants. To calibrate printhead offsets with these grids:

1. Mark a corner of the petri dish to remember the orientation of the print.
2. Ideally, label each printheads' materials with different colors to distinguish between their outputs.
3. Print the structure.
4. Measure the difference between the expected distance between each line (grid spacing) with the actual spacing in both the X and Y direction.
5. Change the printhead offset parameters with the difference that was calculated in step 4.

For alternative calibration procedures, see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6119900/

Below is a demonstration of how to calculate the error in printhead offset. Notice that the X offset is increased by the offset error because the error was measured in the positive X direction. Likewise, the Y offset was decreased by the error because the error was measured in the negative Y direction.





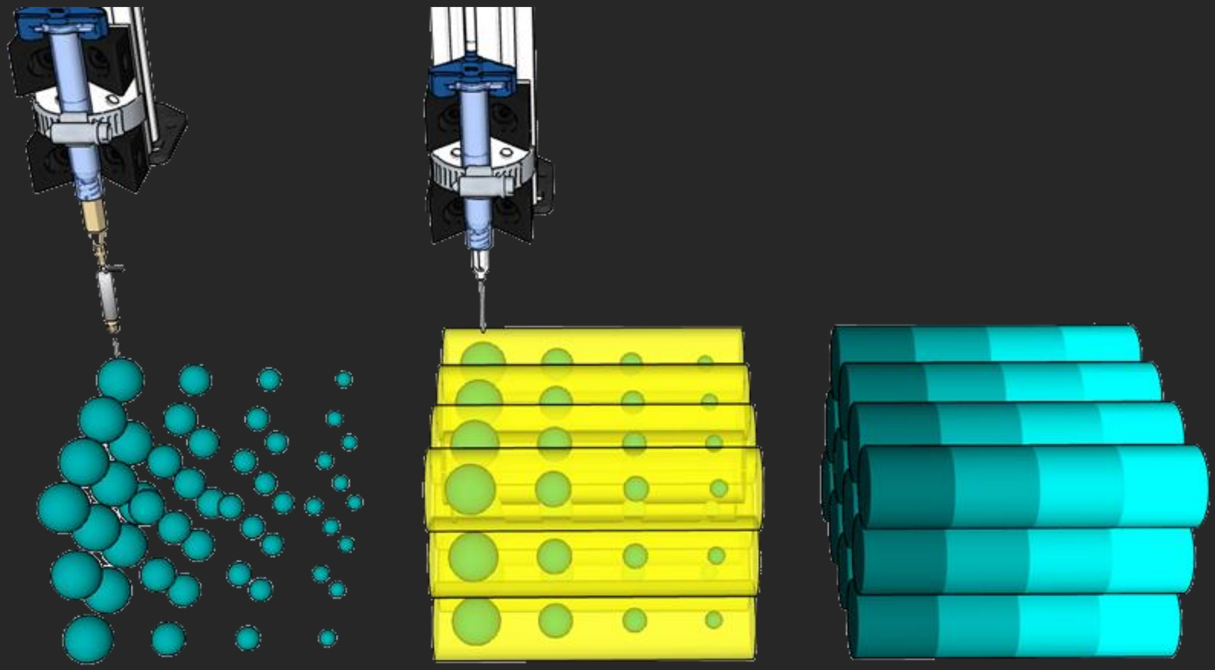
Calibrating more than two printhead offsets or calibrating Z offsets in this manner will require generation of custom patterns for quantification.

Concentration Gradients Embedded in Hydrogels

Concentration gradients are special feature of the ModiPrint system. It enables patterning of small molecule gradients either standalone or embedded within hydrogels. Dispensing concentration gradients without hydrogel embedment is straight forward as it only requires a microdispensing inkjet and an aqueous material. The concentration gradients explanation in the material settings section of this user guide can explain this operation.

Concentration gradients embedded within hydrogels were designed to work with FRESH support slushie. When dispensing gradients within the FRESH support gel, movement of the nozzle creates temporary gaps in the support bath which spreads the small molecules along the toolpath. A hydrogel must be printed along the same path where concentration gradients were printed. The hydrogel must mix with the small molecule gradient before crosslinking and entrapping the small molecules within.

The small molecule gradient is intended to be generated with the microdispensing inkjet. While the microdispensing inkjet is best suited for low volume droplet dispensing, pneumatic extruders and motor-driven printheads can be programmed to generate gradients as well. The hydrogel is intended to be dispensed by the pneumatic extruder, though other printhead types can be programmed for extrusion as well.

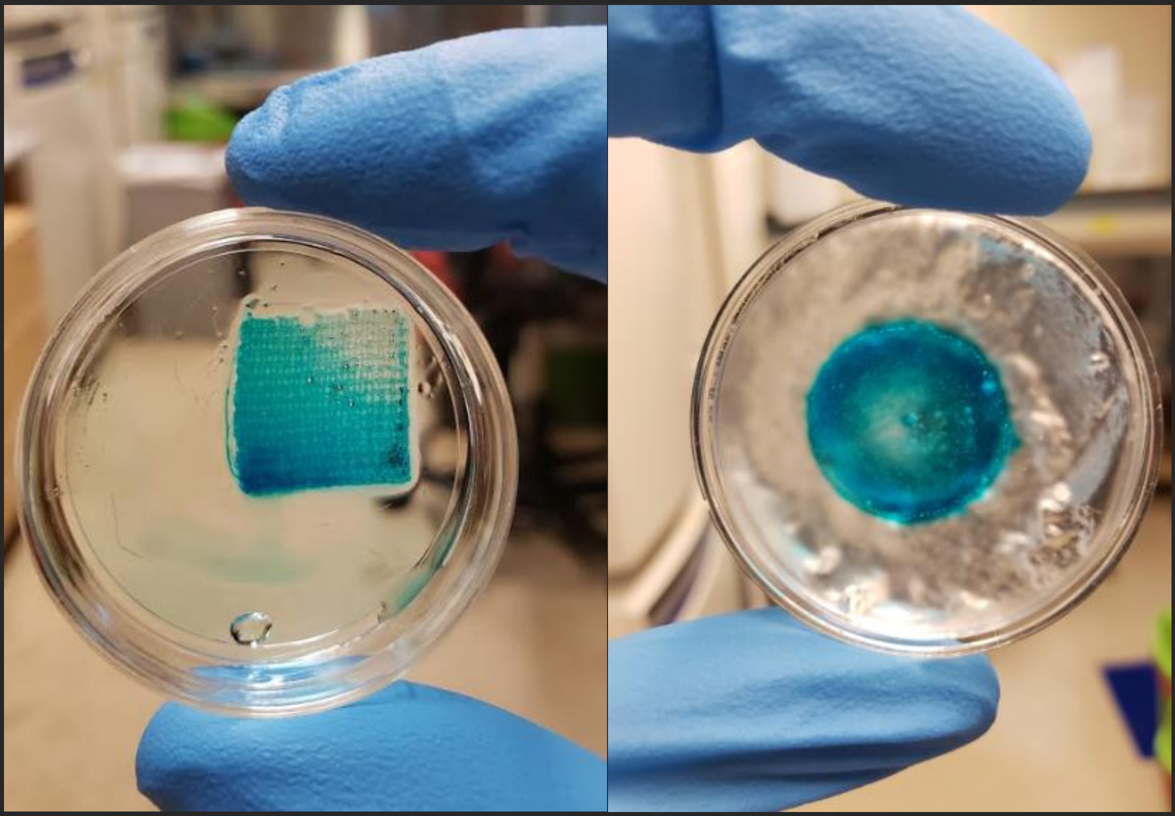


Included in the "Tool Path Samples" folder are two example files for generating small molecule gradients embedded within hydrogels. One file generates a concentration gradient embedded within a 16mm disc. Another file generates a concentration gradient embedded within a 15 mm grid.

Notice that the g-code files used in this application are simply two overlapping tool paths. The shape and strength of the gradient and other print settings would be set in the material settings. The first printhead needs to be a microdispensing inkjet and would correspond to the g-code ID "T3" for these g-code files.

The second printhead will need to extrude the hydrogel and would correspond to the g-code ID "T1". Note that spread of the aqueous solution is likely greater than the filament diameter of the hydrogel. The aqueous solution's tool path XYZ spacing in this likely case should be larger than the hydrogel's XYZ spacing. Also note that printhead offsets do not need to be calibrated too carefully for this operation as the hydrogel and small molecule gradients do not require precise overlap.

Below are images of the prints of these two g-code files. Both geometries were printed with a point gradient shape and the print origin set as the gradient location. Gradients are exponential with gradients strengths of 35% per mm and droplet interpolate distances of 2 mm. 2% alginate was crosslinked over a gradient of blue food coloring. The image on the left is of a hydrogel with embedded gradients. The image on the right is of a concentration gradient suspended within FRESH gel.



G-code files of concentration gradients embedded in hydrogels consist of overlapping toolpaths which Slic3r does not support. Different XYZ spacings are likely needed for the small molecule gradient and hydrogel, so the user will need to generate two different g-code files then combine them.

Here are the instructions for generating a .gcode file for a small molecule concentration gradient embedded in a hydrogel for any arbitrary geometry. This will example a cylinder that is 4 mm (outer diameter) x 1 mm (inner diameter) x 20 mm (length) with a lengthwise concentration gradient. All files used in this example can be found in the "Cylinder Gradient" folder.

1. Generate an arbitrary geometry with CAD.
2. On Slic3r, generate separate .gcode files for the small molecule concentration gradient and another for the hydrogel using the same .stl file.

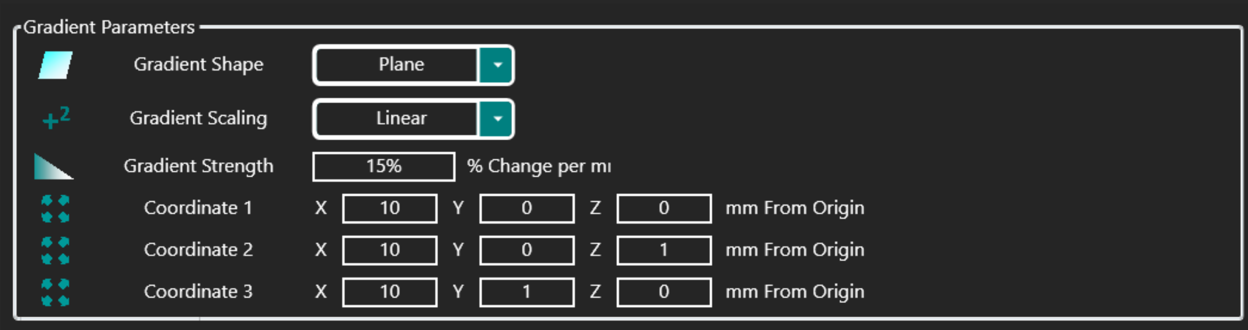
   a. For the cylinder example, 500 µm XYZ pitch is set for the concentration gradient. 200 µm XYZ spacing is set for the hydrogel. XY spacing corresponds to the nozzle diameter parameter in Slic3r and Z spacing corresponds to layer height.
   b. Ensure the different printheads are represented in each g-code file. Different printheads are set by changing the extruder in setting in Slic3r.

3. Append these two lines to the end of the gradient .gcode file:

   G90 ; Absolute coordinates
   G00 X0 Y0 ; Return to the origin.

   Then append the hydrogel .gcode file after these two lines.
   a. .gcode files can be viewed and edited on Notepad or any basic text editing software.

4. In the appropriate "Material Settings" tab in ModiPrint's desktop program, set the parameters to generate a concentration gradient.
   a. In the cylinder example, parameters are set as such (image below). The gradient shape and coordinates are set such that the printer will generate a lengthwise gradient. Since the .gcode files set the origin at the center of the cylinder and the cylinder's length is oriented in the X direction, these settings will place the gradient's origin plane at the end of the cylinder length.



Below is an image of a printed alginate cylinder of this example with a gradient of blue food coloring. To demonstrate that the cylinder is hollow a syringe is perfusing red food coloring through the center.

Concentration Gradients and Hydrogel Resolution

The act of maneuvering the microdispensing inkjet through the FRESH hydrogel slushie degrades the support bath's integrity. Therefore, the act of embedding a concentration gradient decreases the resolution of the hydrogel filament. Dispensing a high volume of aqueous solution into the support bath is particularly damaging. So be aware not to set the gradient strength or valve open time too high.

For more details about this phenomenon, see our paper (especially the supplemental section).

Contact Information & Links

ModiPrint Creator:
Edwin M. Shen
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ModiPrint Website:
www.modiprint.com

Documentation Files:
https://ucmerced.box.com/s/g8jh4kwsjujkn59mi2j91hqzhw5ar8gg

Desktop Program Source Code:
https://github.com/eshen2210/ModiPrint-Desktop

Lab Website:
http://kara-mccloskey.squarespace.com/

Publication:
https://www.sciencedirect.com/science/article/pii/S2405886620300403