

Tissue Scribe™

3D Cultures



USER MANUAL Version 3.0

- ⊗ Product pictures are for reference only.
- ⊗ Color(s), size, parts, and interface may vary.
- ⊗ Preheating is not recommend with the use of cells.
- ⊗ This manual and specifications described herein are subject to change without notice.
- ⊗ All trademarks are the sole property of their respective companies.
- ⊗ This product does not provide a User Interface but reference many that are helpful.
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SAFETY WARNINGS & PRECAUTIONS

- » This device is HOT! Do not touch hot surfaces.
- » The extruder holder can be very HOT! Do not touch when hot.
- » The build platform can be very HOT! Do not touch when hot.
- » Do not touch any moving parts during printing.
- » Do not set extremely high temperatures, this device is develop to provide a cell friendly environment of 37°C. Do not exceed 70°C.
- » Keep the printer and all accessories out of reach of children.
- » This is not a commercial product, it's a Research and Development device and should only be used for research and development only!
- » Do not use on humans or animals.
- » Do not remove or disconnect the USB cable when printing from a computer.
- » Do not pull or twist or modify the black cable at any time.
- » Do not force or tear anything during unpacking or setup.
- » Ensure the printer is turned off and unplugged when not in use.
- » Do not performance any modifications or repairs by yourself.
- » Do not open the printer.
- » Do not install this device on an unstable surface where it could fall and cause wither personal injury or damage to the device and/or other equipment.
- » Do not submerge the device in any liquid.
- » Do not subject the product to extreme force, shock, or fluctuation in temperature or humidity.
- » This device is intended for indoor use only.
- » Do not expose this device to water or moisture of any kind. Do not place drinks or other containers with moisture on or near the device. If moisture/liquid gets in or on the device, immediately unplug it from the outlet and allow it to fully dry before reapplying power. Do this at your own risk, it is recommended to purchase a replacement if the device is water damage.
- » Use only in a well-ventilated area.
- » Do not use in closed, confined spaces.
- » This device may be used in a bio-hood. Ensure device is sterile before placing into a bio-hood.
- » Device is not sterile upon arrival.
- » Sterilize the printer before using, for biological studies.
- » Prior to operation, ensure the power cord is not damage. Do not use if physical damage has occurred.
- » Never unplug the printer by pulling on the cord. Always grasp the connector head or adapter body.
- » Do not move the printer while it is plug in/power on. Always disconnect power source before moving the printer.

OVERVIEW

Thank you for purchasing this 3D Bio-Printer by 3D Cultures. At 3D Cultures, our aim is to use existing technologies and make by printing affordable. There are tons of new, great, and open source technology today that can aim in the development of 3D Bioprinting. This device/printer is built on a open source platform which requires some skills from the end-user. Since many open source user interface can be used with this printer, finding the right one for use will depend on what works for you. This printer also uses G-codes. If you're comfortable coding, this will be the best options. This device is a cheap options for anyone who wants to get into bioprinting. This device is not, by any means, developed for complex printing.

This printer has a single extruder (bio-head). It uses a standard BD 10mL syringe with interchangeable luer-lock tips. This printer can print a variety of materials including, but is not limited to; gelatin, alginate, chitosan, collagen, etc. The limitation of printing is the material viscosity. Thick materials combined with extremely small tips may prove to be more difficult to print, compared to thinner materials and larger tips.

You can print from a Windows or Mac PC using a USB connection or print from 3D model files stored on a microSD card without the need for a computer connection. This printer is easy to use and setup.



Figure 1. Overview of critical components of the printer.

PACKAGE CONTENTS

Below are a list of contents includes with your purchase. Please contact customer service for replacement of any missing items.

- 1x 3D Bio-Printer
- 1x BD Syringe
- 1x USB Cable
- 1x MicroSD Card
- 1x Luer-lock syringe tip (random size)
- 1x Hex Wrench
- 1x Power Adapter
- 1x AC Power Cord

FEATURES

- ✓ Syringe-based biohead
- ✓ Inter-changeable tips
- ✓ Can print a variety of materials, cells, and biologics
- ✓ Open frame allows for ease of use.
- ✓ Includes a microSD card with Cura, Repetier-Host, User Manual, and Nozzle Setup file.

CUSTOMER SERVICE & TECHNICAL SUPPORT

3D Cultures' Support team is always available to help. If you need any assistance please contact us via email at: 3dcultures@gmail.com

USING THE INTERACTIVE SCREEN SYSTEM

This printer has an interactive screen to help with all your printing needs. The menu system is displayed on a lighted LCD Screen at the front of the printer.

1. The menu selection highlight or pointer is moved by rotating the control wheel to the right of the display.
2. Rotating the control wheel counterclockwise moves the highlight/pointer to the left and up.
3. Rotating the control wheel clockwise moves the highlight/pointer to the right and down.
4. Rotating the control wheel counterclockwise while editing a value decreases that value.
5. Rotating the control wheel clockwise while editing a value increases that value.
6. Pressing the center of the control wheel will activate the selected menu item or to save an edited value.

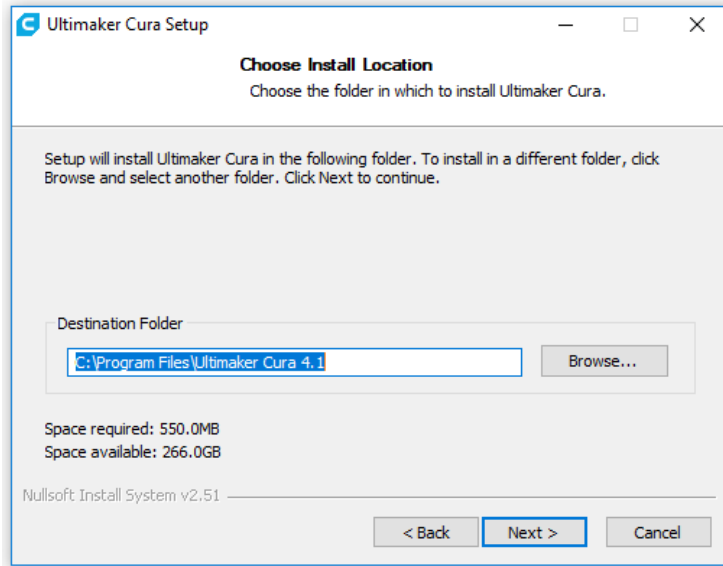
SOFTWARE INSTALLATION

The recommended method of printing is directly from a gcode file on the microSD card. This method does not require a continuous connection to your computer. Gcode files can be downloaded to the microSD card and then loaded into the printer.

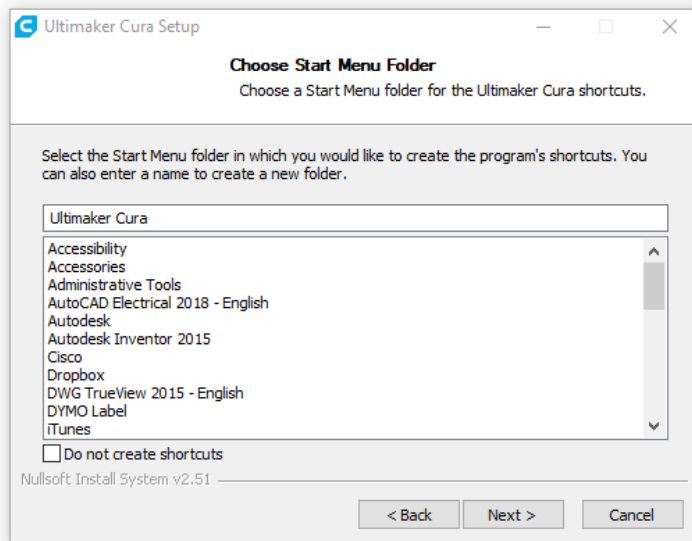
To create your own gcode file(s), you may use an open source program, such as Cura or Repetier-Host (comes pre-installed within the MicroSD Card). These programs use M200 models (typically .STL or .OBJ files) and, using machine specific information, generate a gcode file with detailed instructions for the printer to create the selected model. We recommend start with Cura since it includes a Slicing Engine. However, most open source software will work.

Installing Cura for Windows

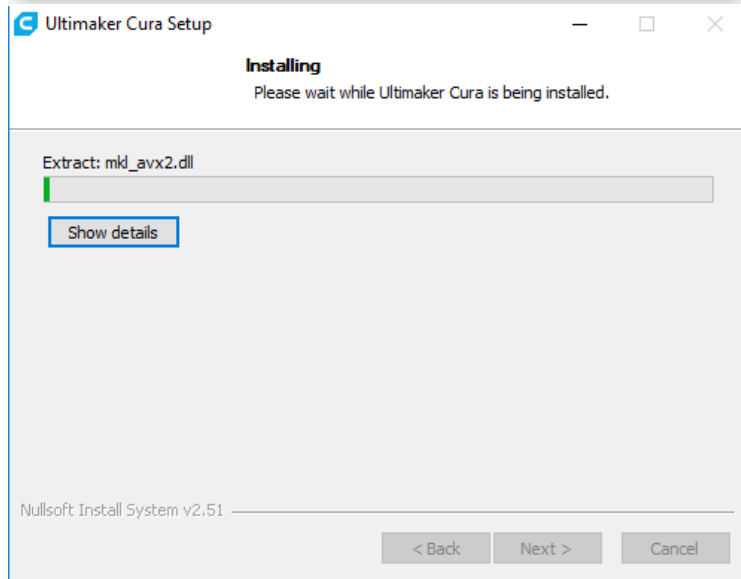
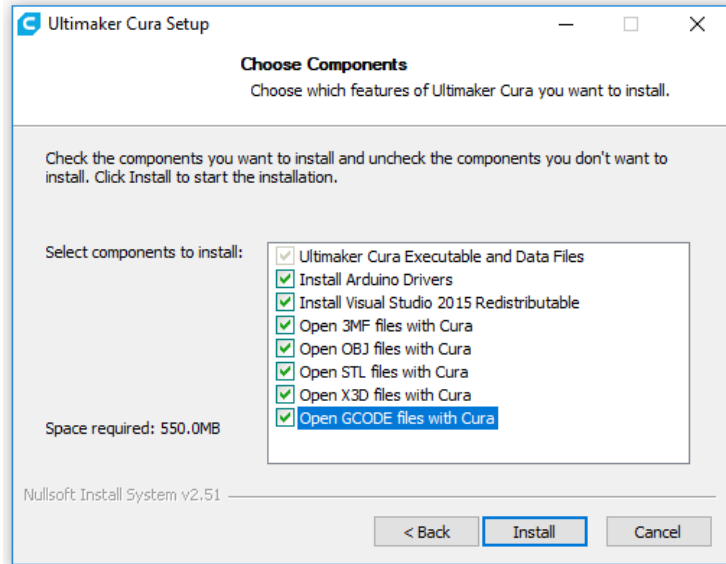
1. Use a card reader to read the contents on the included MicroSD card. Locate and double-click the **Cura_4.1.exe** file to start the setup program.
2. The Setup Wizard will start the installation process. Read the instructions, then select Next to continue.
3. Review the License Agreement.
4. After agreeing. You may choose a new location for the program files or accept the default (recommended). Click **next** to continue.



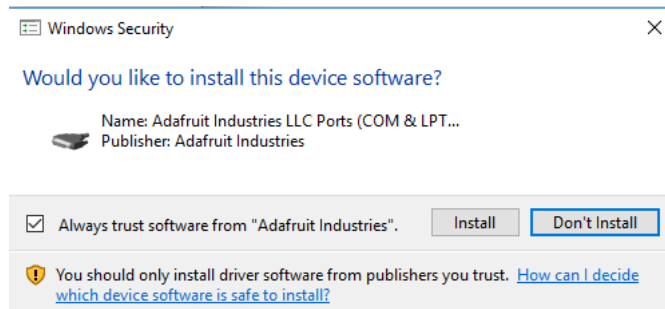
5. You may select a Start Menu folder or leave it blank (recommended) and then select next.

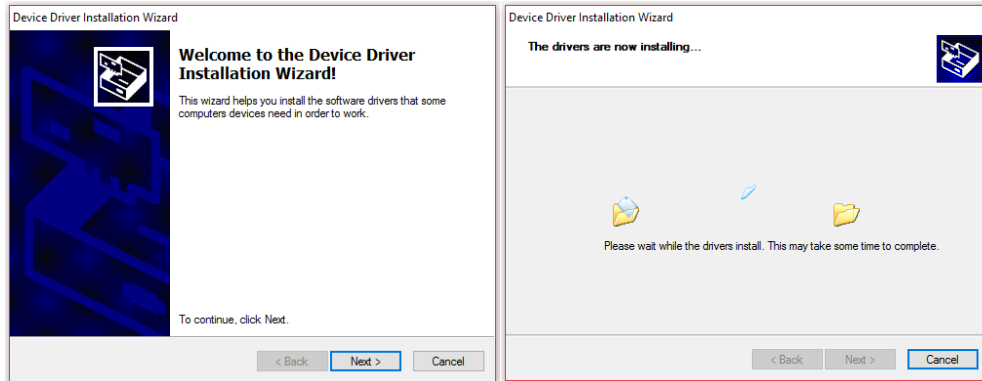


6. Ensure that all boxes are checked, then click **install** to continue.

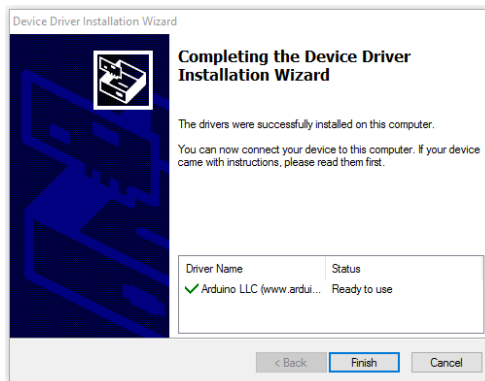


7. Once the files have been extracted and Cura installation is complete, the driver installation wizard will launch. Click **INSTALL** to continue.



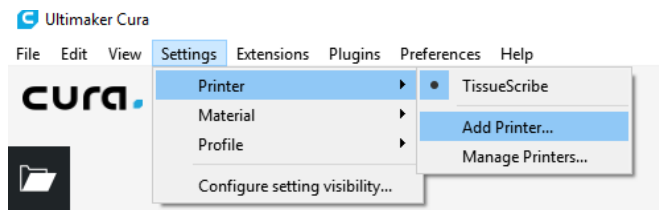


8. Click **Finish** to complete the driver installation.

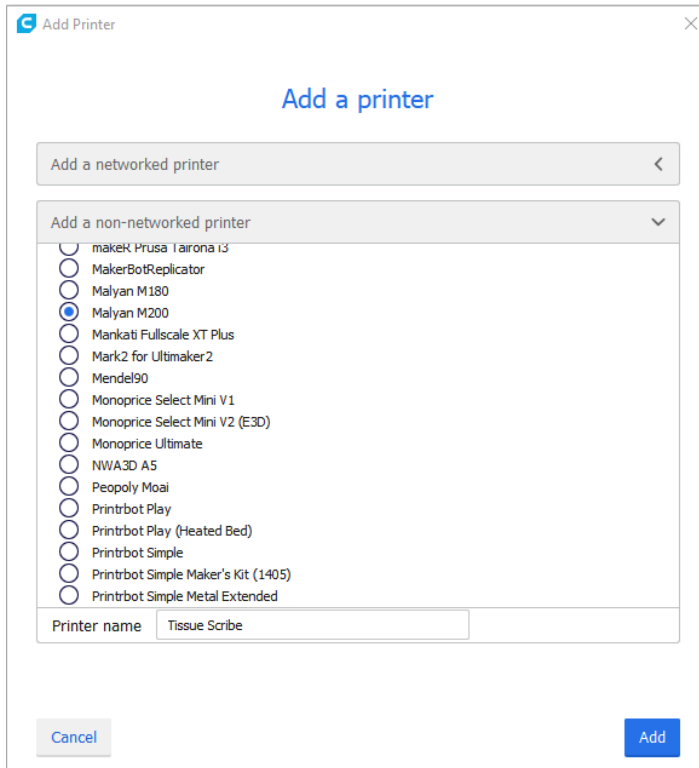


9. Click **next/close/finish** to continue and start the software.

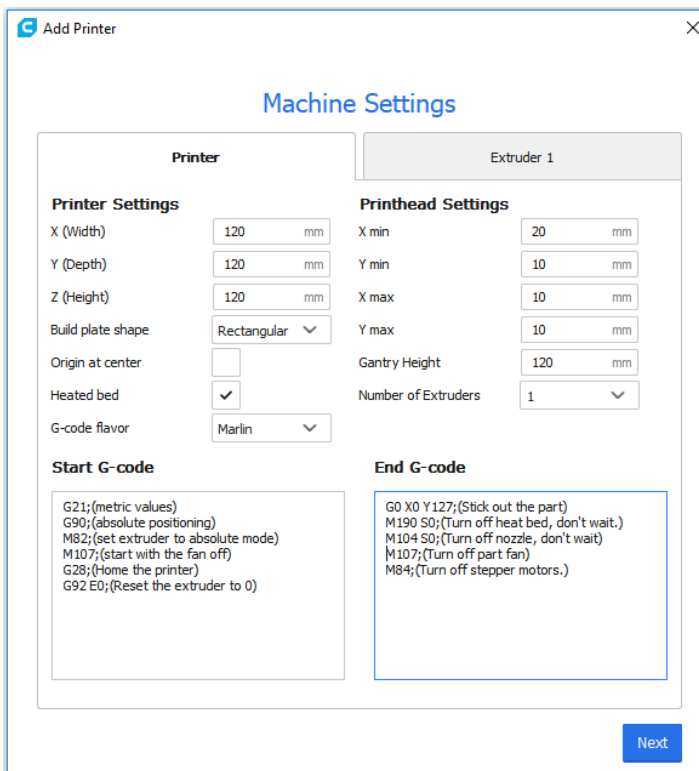
10. Wait for Cura to finish launching. If this is the first time you have run Cura on this machine, Cura will automatically launch the **First time run wizard**. Otherwise, you will need to select from the drop down menu: **Settings>Printer>Add Printer**.



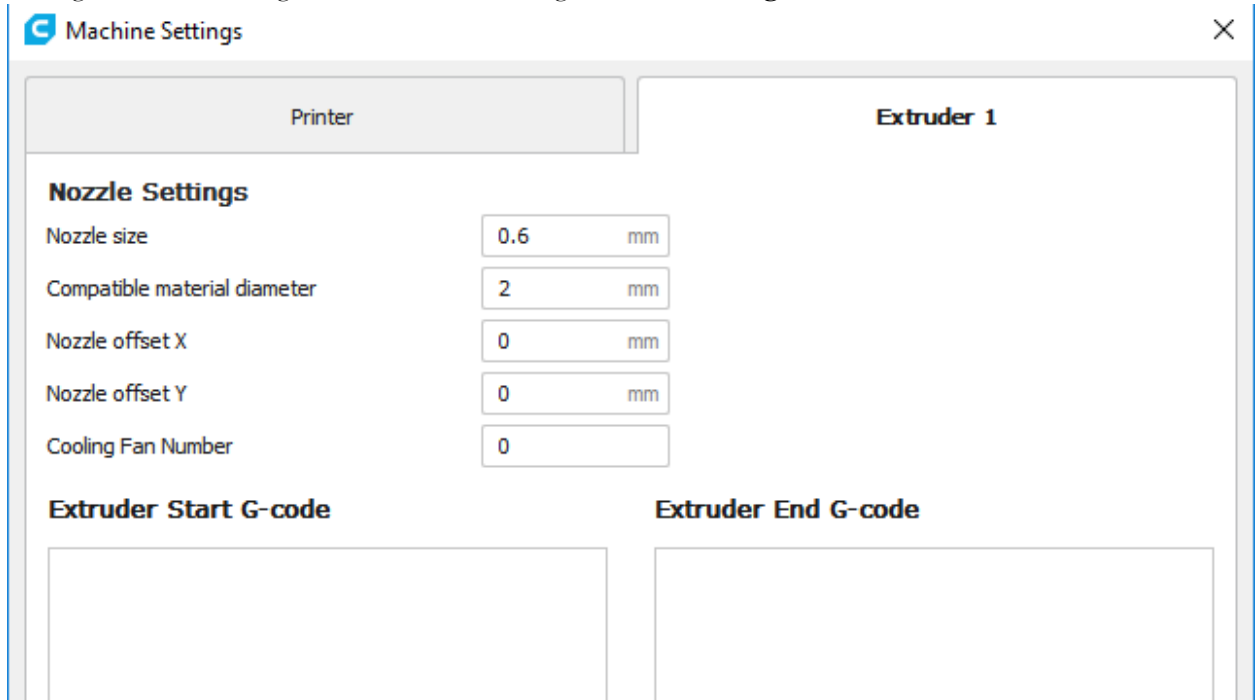
11. Select “**Add a non-networked Printer**”, a list will expand. Go to **Other** and select **Malyan M200**, then Enter **Tissue Scribe** for Printer name, then select **Add**.



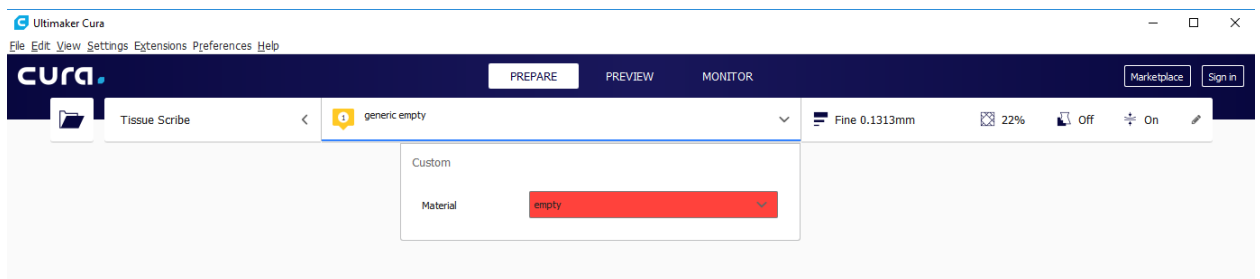
12. Machine Settings will pop-up, your values should be the same as the figure below for the Printer Tab.



13. For the Extruder Tab, this controls your extrusion rates. Please note that the Nozzle size will be used to determine the flow rate/extrusion rate. This is a function of the material viscosity and the nozzle tip use. You may need to change this value until you've established the correct extrusion rate. To setup, let's start let's use the values below. You can always change them later by going to: Settings>Printer>Manage Printers, then selecting **Machine Settings**. Then click finish or close.



14. The program will finish launching and leave you on the main screen. On the Top, next to the printer's name, you should be able to change your print parameters. For material it may say "EMPTY":



You do not have to create a material profile to start using the printer, you can continue with this as blank. Select the **Basic** tab, then change the settings to match those in the accompanying image for PLA filament. (PLA is generic since these interface are not set for bio-materials. You will have to manually set the temperature and extrusion rate).

Note: The fill density, support type, platform adhesion types, temperature, etc options should be set according to the needs of the model you are printing.

The fill density will determine the overall strength of the finished object and the internal architecture. It is not recommended to use support with biological model. Unless absolutely critical and need, this will depend on the user.

Since there are no set standards in bioprinting, the selection of the features/settings below is up to the user. Take notes of what works for your printing needs. Note that it will require a few trials to get it right. It is recommended to test your printing before printing the actual experimental sample.

The extrusion rate is controlled by changing the values of: Filament Diameter, Flow, and Nozzle size.

The screenshot displays the TissueScribe software interface. At the top, the 'Material' is set to 'PLA'. Below this, there is a 'Check compatibility' link. The 'Print Setup' section is currently set to 'Custom' mode. The 'Profile' is 'Fine - 0.13125mm'. A search bar is present below the profile selection. The settings are organized into several expandable categories: 'Quality' (Layer Height: 0.1313 mm), 'Shell' (Wall Thickness: 0.8 mm, Top/Bottom Thickness: 0.8 mm), 'Infill' (Infill Density: 100%, Gradual Infill Steps: 0), 'Material' (Printing Temperature: 37 °C, Build Plate Temperature: 37 °C, Diameter: 10 mm, Flow: 100%, Enable Retraction: checked), 'Speed' (Print Speed: 10 mm/s, Travel Speed: 120 mm/s), 'Cooling' (Enable Print Cooling: unchecked), 'Support' (Generate Support: unchecked), 'Build Plate Adhesion' (Build Plate Adhesion Type: None), and 'Special Modes'. The 'Diameter' field is highlighted in orange.

15. (Hamid, Q. 2014, Combining fabrication and surface modification techniques to develop cell-laden microfluidic devices) have validate material deposition as a function viscosity, pressure, and tip size: *Material from the printer head is governed by the material viscosity, applied pressure, length of the nozzle tip, and the radius of the nozzle. To accurately input the process parameters needed for a specific filament size, Poiseuille's Law (Equation 0-1) is utilized.*

$$\frac{dV}{dt} = \frac{\pi}{8} \left(\frac{R^4}{\eta} \right) \left(\frac{P}{L} \right) \quad 0-1$$

where R is the radius of the nozzle, P is the applied pressure at the inlet (inner orifice of the nozzle) of the nozzle, L is the length of the nozzle, and η is the viscosity of the material. The pressure, P of the photo-polymer head is calculated with Equation 0-2 where F is the applied force and d is the diameter of the syringe. Experimentally derived; the maximum force at the maximum feed-rate of 1000 mm/min for a 10 mL syringe of the photo-polymer head is 110 lbs, while the maximum force at the minimum feed-rate of 1 mm/min of the photo-polymer head is 133 lbs.

$$P = \frac{F}{2\pi d^2} \quad 0-2$$

The force required for motion is calculated using Equation 0-3, where A is the cross-sectional area of the syringe, l is the length of total volume of material in the syringe, and v is the motion speed of the syringe's plunger. This force is directly proportional to speed, greater forces will result in faster flow rates.

$$F = \eta A \frac{v}{l} \quad 0-3$$

The motion speed of the syringe's plunger is calculated using Equation 0-4 where P_1 is the applied pressure and P_2 is the pressure at the end of the syringe (where the nozzle is connected), r is the inner radius of the syringe. The speed at any point is proportional to the change of pressure per unit length (pressure gradient).

$$v = \frac{P_1 - P_2}{4\eta L} r^2 \quad 0-4$$

16. Click **Advanced** Tab, then change the settings to match those in the accompanying image to get started. These values are for reference only. You should change as needed to get a proper print of your material/construct.

Basic	Advanced	Plugins	Start/End-GCode
Retraction			
Speed (mm/s)	<input type="text" value="10"/>		
Distance (mm)	<input type="text" value="0.1"/>		
Quality			
Initial layer thickness (mm)	<input type="text" value="0.3"/>		
Initial layer line width (%)	<input type="text" value="100"/>		
Cut off object bottom (mm)	<input type="text" value="0.0"/>		
Dual extrusion overlap (mm)	<input type="text" value="0.15"/>		
Speed			
Travel speed (mm/s)	<input type="text" value="50"/>		
Bottom layer speed (mm/s)	<input type="text" value="5"/>		
Infill speed (mm/s)	<input type="text" value="1"/>		
Top/bottom speed (mm/s)	<input type="text" value="1"/>		
Outer shell speed (mm/s)	<input type="text" value="1"/>		
Inner shell speed (mm/s)	<input type="text" value="1"/>		
Cool			
Minimal layer time (sec)	<input type="text" value="5"/>		
Enable cooling fan	<input type="checkbox"/>	<input type="button" value="..."/>	

17. Finally, using the included Micro USB cable, plug one end into the usb port on the printer, then plug the other end into an available USB port on your computer. Watch the right hand corner of the screen. A bubble will appear in the lower right corner of your computer which is telling you that windows is installing the necessary printer drivers. Click the bubble and verify the drivers have been properly installed.
18. You may modify the start and end code of your gcodes by selecting the Start/End-GCode tab. This area is used to setup beginning and ending constraints of your print. For example, if you're manually set the starting position of your print job, you will not want to home the axis first and then go to that position and print. In this case you will remove the homing codes and use the M92 Code to set the current value of the current position. Hence the printer will use those value.

```

Basic | Advanced | Plugins | Start/End-GCode
start.gcode
end.gcode

;Sliced at: {day} {date} {time}
;Basic settings: Layer height: {layer_height}
;Print time: {print_time}
;Filament used: {filament_amount}m {filament_color}
;Filament cost: {filament_cost}
;M190 S{print_bed_temperature} ;Uncomment to
;M109 S{print_temperature} ;Uncomment to
G21 ;metric values
G90 ;absolute positioning
M82 ;set extruder to absolute mode
M107 ;start with the fan off

G28 X0 Y0 ;move X/Y to min endstops
G28 Z0 ;move Z to min endstops

G1 Z15.0 F{travel_speed} ;move the platform

G92 E0 ;zero the extruded length
G1 F200 E3 ;extrude 3mm of filament
G92 E0 ;zero the extruded length
G1 F{travel_speed}
;Put printing message on LCD screen
M117 Printing...

```

This is an example of a startup code for printing at the point in the printer is current at. This sets the current position of the nozzle at X=0, Y=0, Z=0. This code will not home any of the printer's axis.

```

M109 S25.000000
G21 ;metric values
G90 ;absolute positioning
M82 ;set extruder to absolute mode
M107 ;start with the fan off
G92 E0 ;zero the extruded length
G92 X0 Y0 Z0 ;set current position of X Y Z to zero
G1 F1200

```

Now if your current position is not 0, 0, 0, modify the bolded code below to change it. Typically, this can be the value of your starting point of your model, this will be the first line of code when you're generating your g-code. No worries, this value can be entered/modified after you've generated the codes once.

```

M109 S25.000000
G21 ;metric values
G90 ;absolute positioning
M82 ;set extruder to absolute mode

```

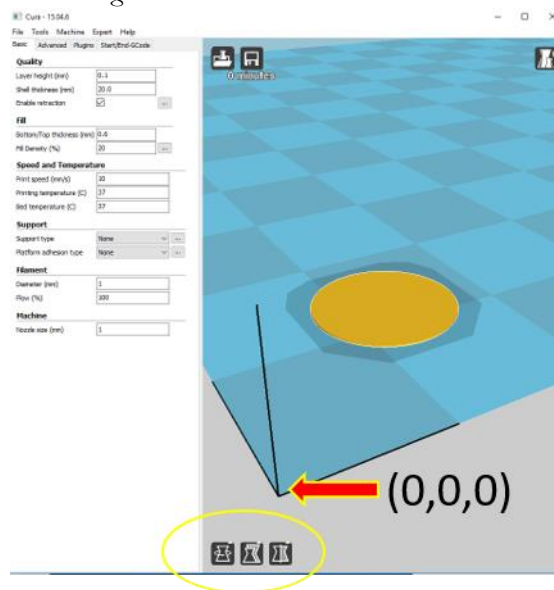
M107 ;start with the fan off
G92 E0 ;zero the extruded length
G92 X0 Y0 Z0 ;set current position of X Y Z to zero
G1 F1200

14 Congratulations, you have installed Cura and connected your PC to your new 3D Bioprinter! Refer to Cura's documentation for operating details and information on the various options available to you.

Loading a CAD MODEL (.stl) into Cura and generating G-codes.

You can also install Repetier-Host from the file on the included microSD card from an internet download. The screenshots below shows the proper settings to use for this bio-printer in Repetier-Host.

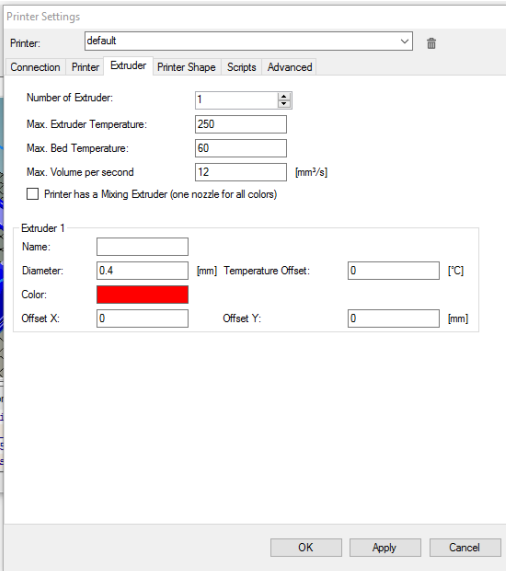
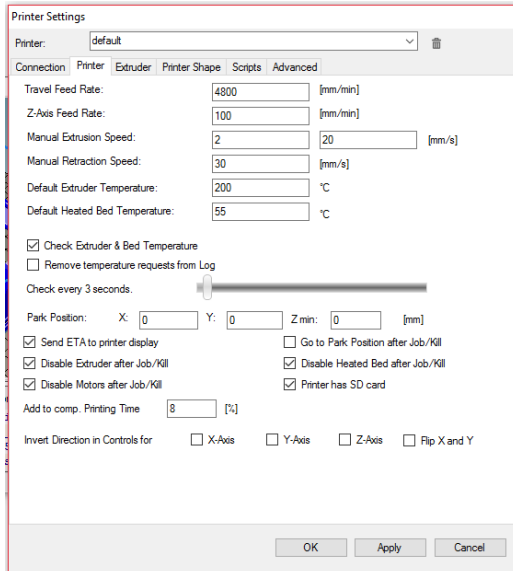
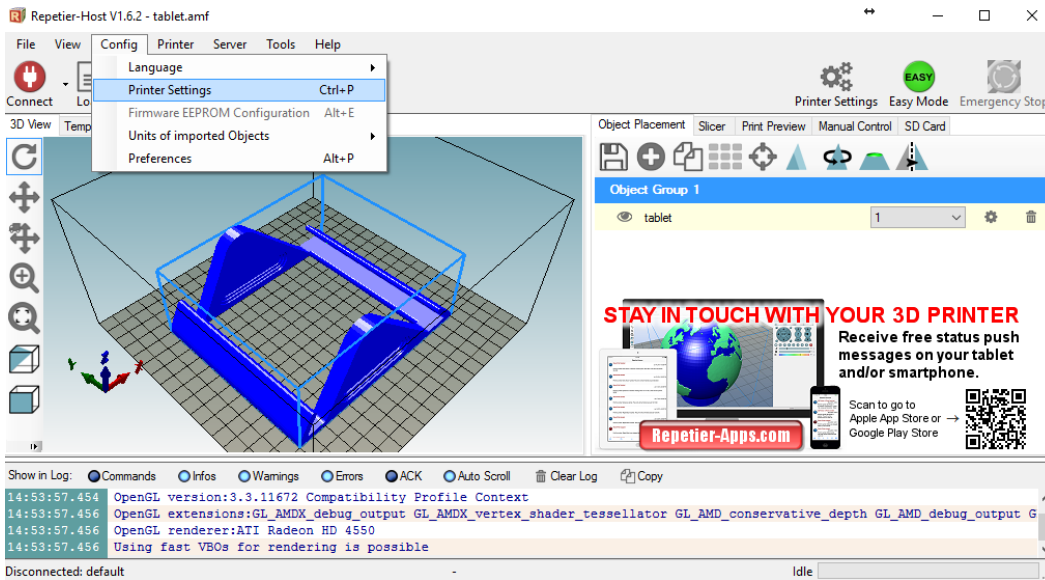
1. Click on the "Load" icon to load your .stl file into Cura.
2. Locate your stl file and load into the software. Cura will place your model in the center of the build. You may move, scale, mirror, and rotate your model to a position and orientation that fits your needs. The icons are at the bottom left. To move, click on the model and then drag.

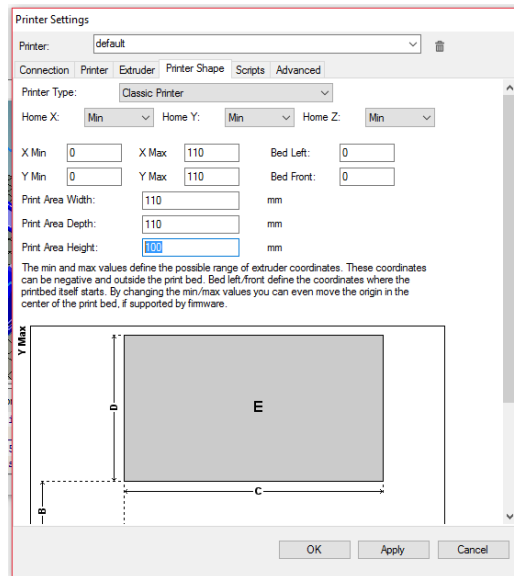


3. Click save toolpath to generate your g-code (located next to the load file icon).

Installing Repetier-Host

You can also install Repetier-Host from the file on the included microSD card from an internet download. The screenshots below shows the proper settings to use for this bio-printer in Repetier-Host.





PRINTER & NOZZLE SETUP

Perform the following steps to prepare the printer for use.

Out of Box Setup

1. Remove the printer from its packaging and place it on a flat, stable surface with plenty of ventilation and a nearby AC power outlet.
2. Remove the any tape or cable ties holding the nozzle.
 - a. If the nozzle is not connected to the motion arm; There are a series on mounting holes for the nozzle (figure below, left), line up to your desired placement and use the hex tool to tighten both screws (figure below, right).
 - b. Future adjustments can be made by moving to different holes or moving the nozzle up and down.



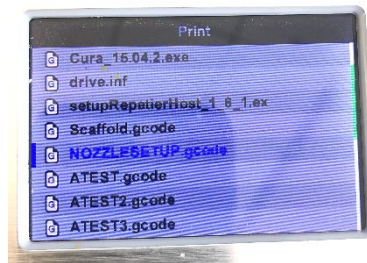
P

3. Ensure that the power switch on the rear panel is in the OFF position (“O” side depressed). Plug the C13 end of the included AC Power Cable into the C14 panel connector on the AC Power Adapter, then plug the other end into a nearby AC power outlet. Press the “I” side of the power switch to power the printer ON.
4. Printer is ready for use. Before using, calibrate the nozzle to the Z zero position.

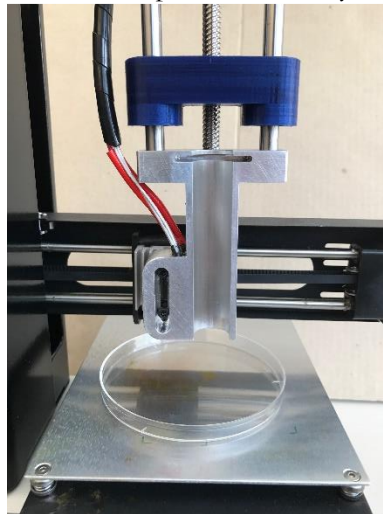
Zeroing the nozzle

Before printing, it is recommended that you zero the nozzle. Zeroing the nozzle ensures that the tip is always set to a reference zero position. Since most tips have a different length you would want to ensure when a tip is changed the reference zero point is calibrated. To set the reference zero, do the following:

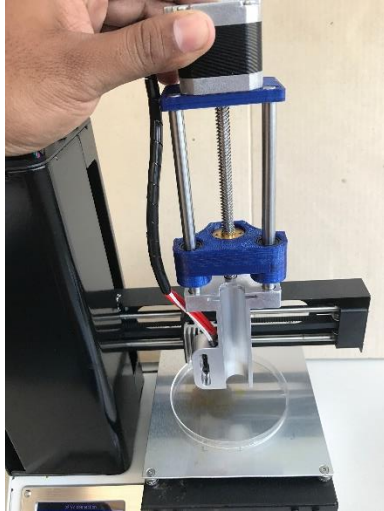
1. Do NOT install the syringe as yet.
2. Insert the microSD card and run the code “NOZZLESETUP.gcode”. This code will move the nozzle to the print platform.



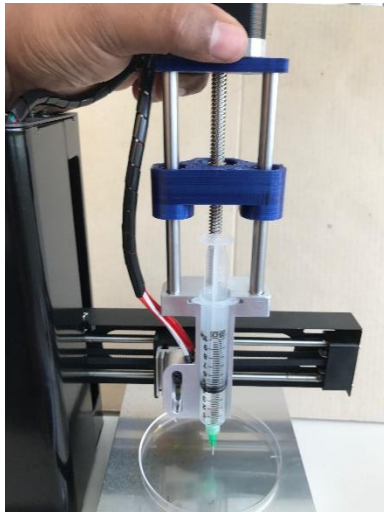
3. If you're printing into a petri dish add it to the platform, directly under the nozzle.



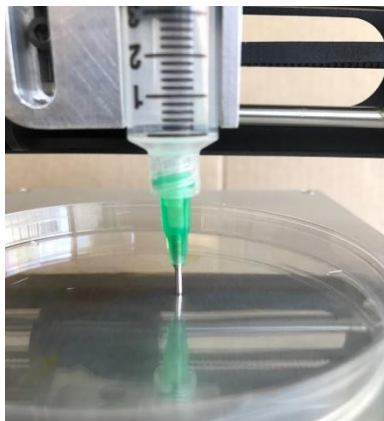
4. Using the hex tool, loosen the two hex screws on the nozzle. DO NOT remove the screws. HOLD THE NOZZLE and DO NOT REMOVE from the heatsink. Two personnel may be required.



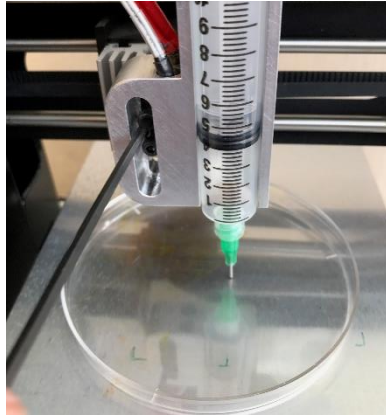
5. Insert the syringe with the tip.



6. Position the nozzle (with the syringe) where the tip barely touches the base of the petri dish.



7. Tighten both hex screw with the hex tool.



8. Using the interactive screen, move the nozzle up.



9. The tip of your nozzle is now at the zero Z position and is ready to print.
10. Note: Ensure the nozzle is at zero position for best printing. If printing into a petri dish, ensure your code takes into consideration the dimensions/parameters of the dish.
 - a. DO NOT Pre-heat if working with cells.

REGULATORY COMPLIANCE

NOTICE FOR FCC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This device is for research and development use only! Any other uses are not permitted. Modifications are not permitted. Any modifications are at your own risk. This device is not intended to be used on humans or animals. This device may use human products or any products approved for research and development purposes only. Any biological materials used, must be from an approved source. Use at your own risk.

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