BIO X Syringe Printhead

User Manual

CELLINK®

Contents

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Package contents

01 Package contents

Item	Part number	Quantity
Syringe Printhead	000000020855	1
BD 3-component 3-mL syringe	D16110021153	3
Locking screw	000000020106	1

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Technical specifications

02 Technical specifications

- Max syringe volume: 2.3 mL.
- Dimensions (height x width x depth):
    - Fully extended: 138 x 37 x 65 mm.
    - Fully contracted: 97 x 37 x 65 mm.
- Weight: 263.6 g.
- Material composition:
    - External surfaces: Aluminum.
    - Internal surfaces: Aluminum.
- Recommended operating temperature: 20-23 degrees Celsius.

NOTE: BIO X should be operated in temperatures between 20-23 degrees. In normal operating conditions, the chamber temperature of the BIO X will rise 2-3 degrees above the ambient temperature and the standard pneumatic printheads will rise above the chamber temperature.

- Running temperature under recommended conditions: 26-32 degrees.
- Heating capacity and rate:
    - Total heating range: 30-65 degrees.
    - Heating rate: Room temperature-40 degrees (10 minutes).
    - Heating rate: 40-65 degrees (12 minutes).

● **Cooling capacity: N/A.**

● **Build volume (X, Y, Z): 128 x 75 mm x (83−A) mm; A represents tip length.**

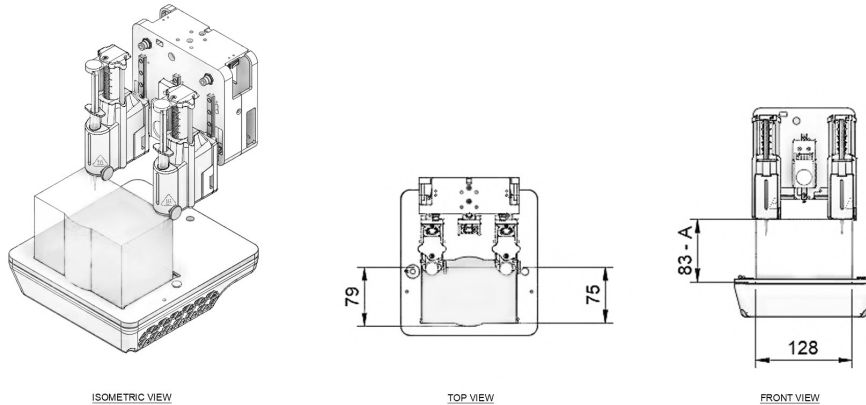

ISOMETRIC VIEW   TOP VIEW   FRONT VIEW

Figure 1: Theoretical maximum build volume as shown from the top and side view when using the Syringe Printhead. Render assumes a nozzle length of zero. Exact build volume depends on the syringe's position in the printhead and the nozzle/needle being used. Build volume may be lower when combining the Syringe Printhead with a printhead that restricts printbox movement. Please consult the printhead manual for the printheads being used for specific limitations.

02 Technical specifications

Table 1: Theoretical build volume based on tips of common lengths. Actual volume may vary based on build plate thickness, size, shape, level, cartridge position and tightness of tip. X, Y and Z are measured from the front left corner of the printbed.

Tip Type	Tip Length	Nozzle/Needle Length	X	Y	Z	Build Volume
Conical	3.2 cm	3.2 cm	12.7 cm	7.5 cm	5.14 cm	489 cm^3
Blunt	2.4 cm	0.635 cm	12.7 cm	7.5 cm	5.94 cm	565 cm^3
Blunt	3.0 cm	1.27 cm	12.7 cm	7.5 cm	5.34 cm	508 cm^3
Blunt	4.3 cm	2.54 cm	12.7 cm	7.5 cm	4.04 cm	385 cm^3
Micron-S	1.9 cm	1.9 cm	12.7 cm	7.5 cm	6.46 cm	615 cm^3

03

Safety information

03 Safety information

3.1 BIO X system warnings

• Please consult the BIO X manual for BIO X-specific and general warnings and safety procedures.

3.2 Syringe Printhead warnings

• Do not submerge the printhead in liquid or use excessive spraying to clean it. Liquid inside the printhead risks damaging the circuitry and motor.
• The LED on the printhead will turn yellow to indicate a warm printhead and red to indicate a hot printhead. Do not touch the printhead when the LED is red. Touching a hot printhead risks serious user injury.
• Do not manually move the printhead mount. Moving the printhead mount manually will damage the motor. If the mount needs to be moved, place the printhead in the loading position described in the Getting Started section.
• Use the proper method to load and unload the Syringe Printhead onto the BIO X printhead mounts. Instructions are outlined in the Getting Started section.
• Do not pull or push down the locking arm of the Syringe Printhead. Improper handling can damage the motor and the movement guides. Use the BIO X system UI to move the arm.
• Ensure that the syringe plunger arm is not cracked or bent before inserting it into the

Syringe Printhead. Inserting a damaged syringe plunger arm can result in syringe failure and possible injury. Replace the syringe at the first sign of wear.

- Tighten the locking dial before printing to ensure that the syringe remains in place during the printing process. Improper tightening can cause the syringe to move and material to dispense improperly.
- Do not place fingers on the printhead while it is in motion. Users risk serious, permanent injury if fingers are caught between the printhead base and the motor arm.

04

Getting started

04 Getting started

NOTE: The printing parameters displayed in this manual might not be accurate. For recommended parameters please consult the relevant bioink documentation.

4.1 Unpacking, installation and initial setup

A. Locking Arm
B. Syringe
C. Printhead Body
D. Locking Knob

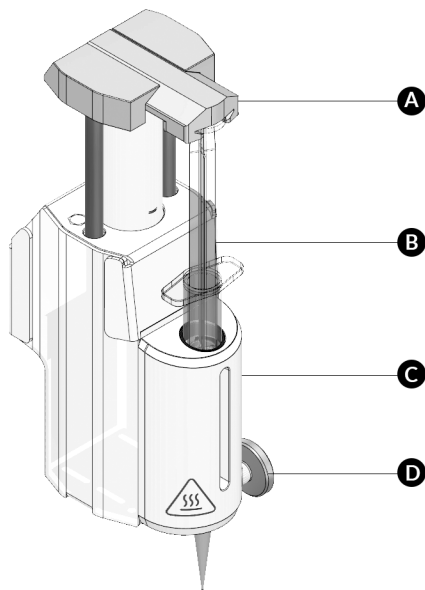**Figure 2:** Components of the Syringe Printhead.

1. Open the package. Remove the printhead and other components.
2. To insert the printhead, align the printhead without a syringe above the desired printhead mount (Figure 3A) and push downward using one hand. Secure the bottom of the printhead mount with your opposite hand to avoid straining the motor (Figure 3B).
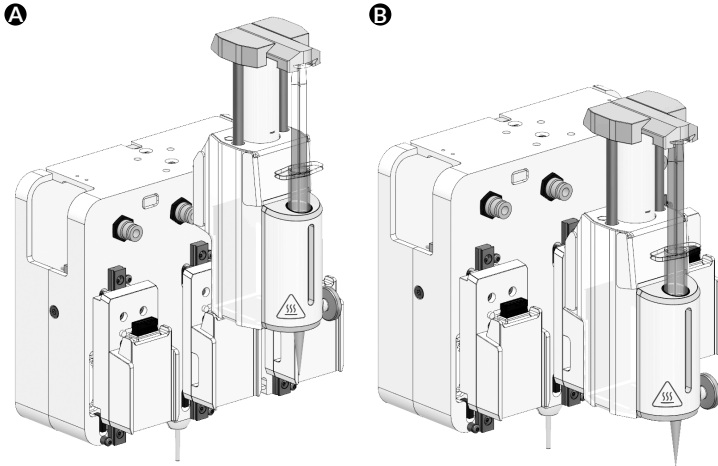
Ⓐ   **Ⓑ**

Figure 3: Inserting the Syringe Printhead into the BIO X printhead mount.

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3. Go to the Utilities menu.

4. Go to the Printer menu and enable "Home to back" to allow the system to measure the Y-axis (Figure 4).

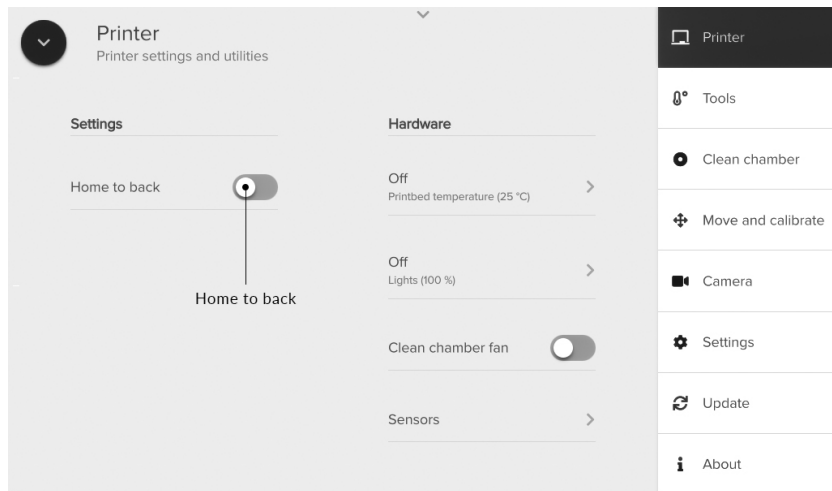5. Go to the Move and Calibrate menu. Home the BIO X system.

Figure 4: Calibrating the BIO X system for the Syringe Printhead.

6. Go to the Tools menu.

7. Lower the Syringe Printhead into the active position (Figure 5).
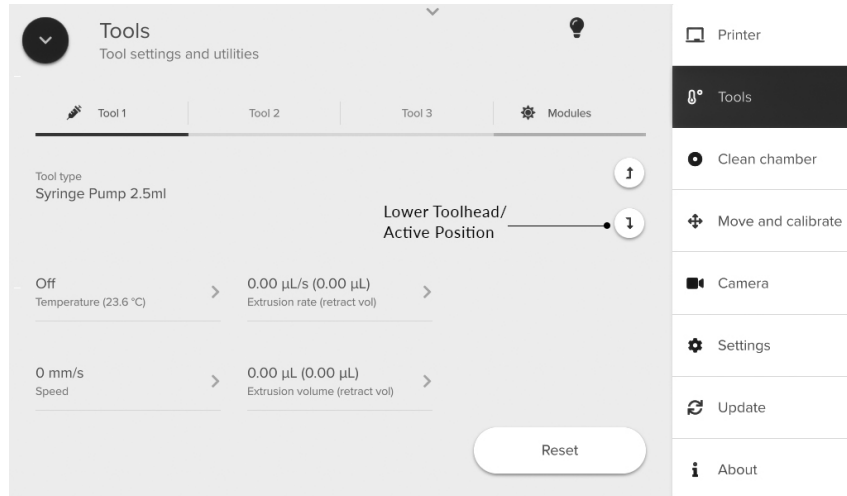

Figure 5: Lower the Syringe Printhead into the active loading position.

8. Under the Tools menu, open the Syringe Printhead parameters (Figure 6A). Fully retract the Syringe Printhead using the Retract Fully function (Figure 6B).
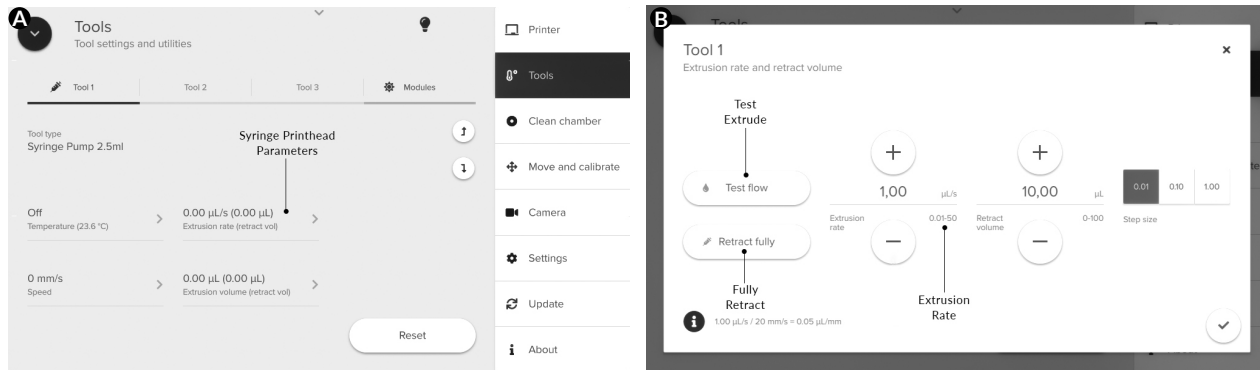

Figure 6: (A) Accessing the Syringe Printhead parameters.
(B) Retracting and extruding the printhead.

9. Fill a 3-mL BD syringe with your desired bioink to a maximum volume of 2.3 mL. Attach a nozzle or needle to the syringe.

NOTE: Remove any bubbles in the bioink before use. Trapped bubbles are a major source of dispensing errors particularly when a precise volume is required.

10. To insert the syringe into the printhead, push the syringe plunger lock to the back (Figure 7A) and load the syringe into the printhead (Figure 7B). Set an extrusion rate and press the test extrude button to lower the locking arm until it is in line with the end of the syringe (Figure 6B). Pull the locking arm forward to secure the syringe plunger (Figure 7C).

NOTE: If the printbox is too far forward, you may need to move it backward before loading. You can do this using the Move and Calibrate menu.

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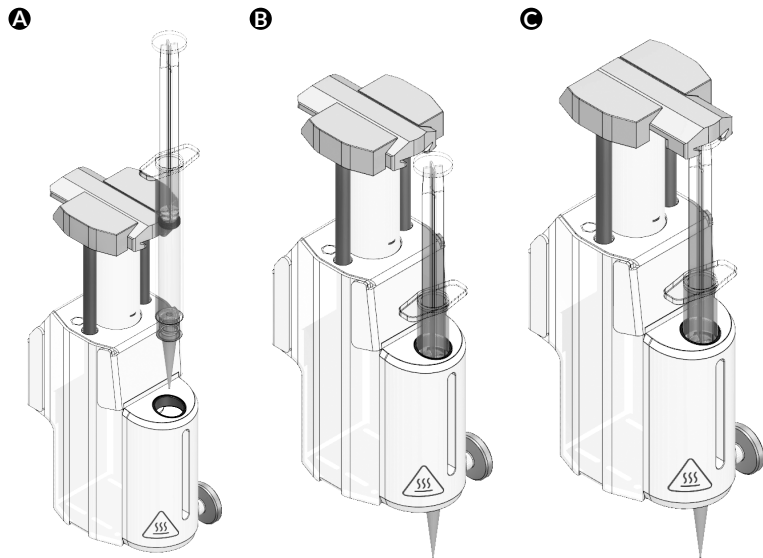
Figure 7: Loading a syringe into the Syringe Printhead.

11. Tighten the syringe locking knob at the base of the printhead.
12. Set the retract volume; 10-25 μL is a good starting point for most bioinks. The retract volume will retract the syringe by the volume you set to stop the flow when extrusion is stopped (Figure 8). See the printing guidelines below for more information.
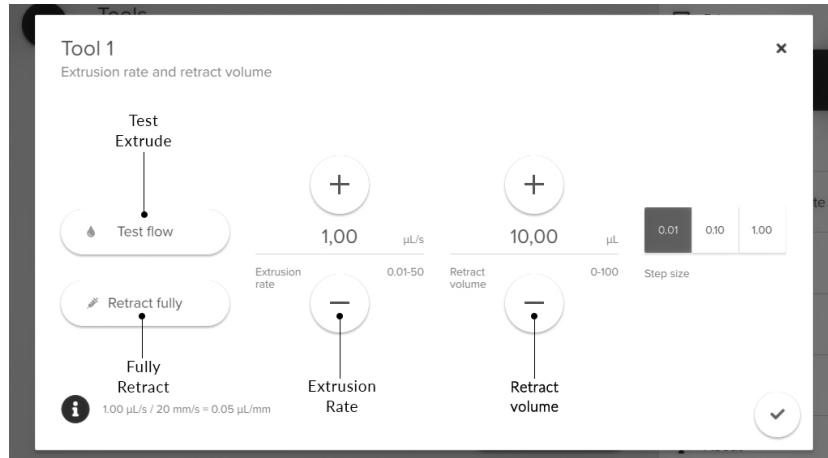
Figure 8: Setting the retract volume. This parameter is necessary to stop flow when extrusion is stopped.

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4.2 Your first bioprint

1. Prepare the printhead and load the bioink into the syringe as detailed in Section 4.1.
2. Select Bioprint from the Start menu (Figure 9).
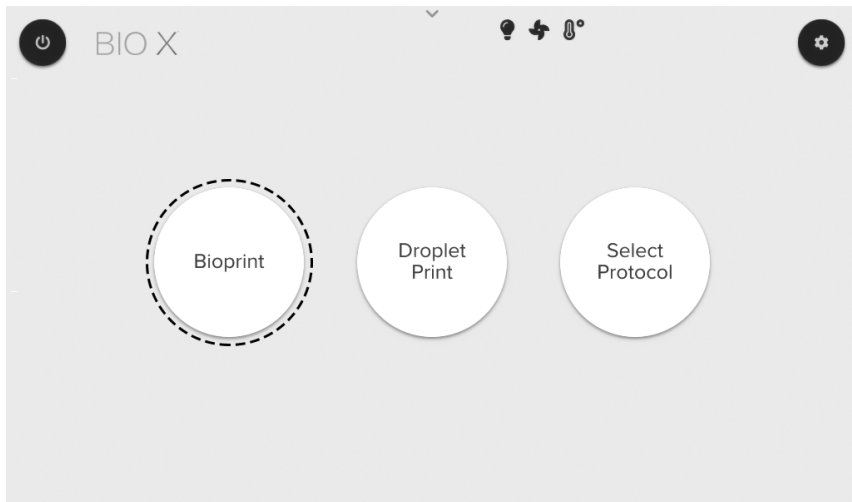
Figure 9: The Start menu. Select Bioprint to proceed to set up a print.

3. Select an STL file (filename.stl) or G-code file (filename.gcode) from the Model menu
(Figure 10). Proceed to the next menu by selecting Surface along the bottom of the screen.

Figure 10: Selecting an STL file from the Model menu.

4. Select a surface to print on (Figure 11). Proceed to the next menu.
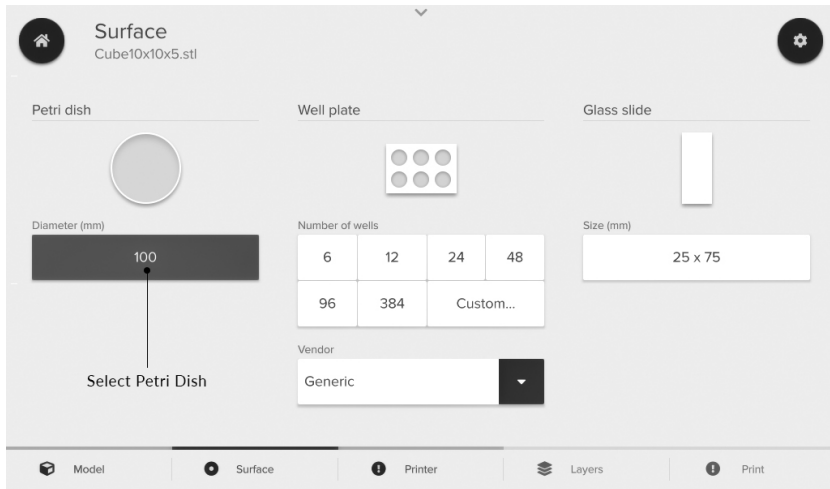
Figure 11: Select a print surface from the Surface menu.

5. Select the printheads you have setup. Ensure that Syringe Pump 2.5 mL is selected under the printhead type (Figure 12).
6. Enter your printing parameters for the printhead (Figure 12). Proceed to the Layers menu. Parameters necessary for the Syringe Printhead include:

- Extrusion rate.
- Retract volume.
- Print speed.
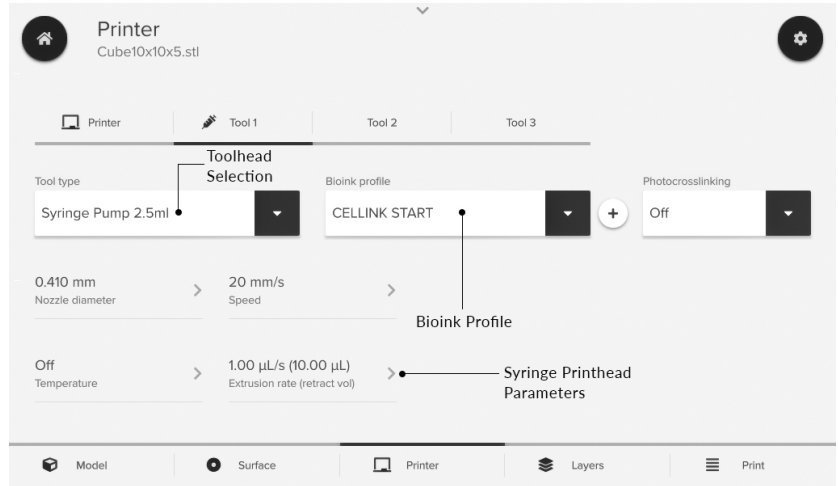
Figure 12: Printing parameters on the Printer menu.

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7. Assign the enabled printhead to the respective layer characteristics and proceed to the next menu (Figure 13).
8. Prime the nozzle/needle and test bioink flow by pressing Drop next to the pressure settings (Figure 14). Press Print to proceed to the calibration page.
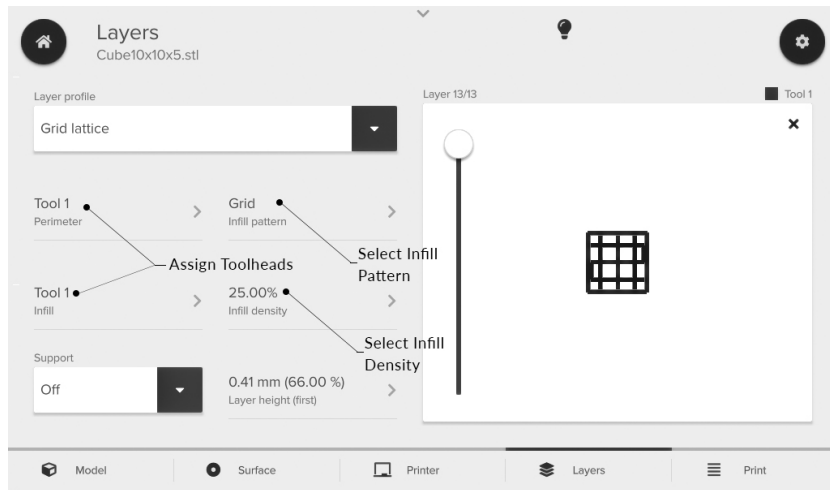
Figure 13: Layer menu. Toolheads can be assigned to respective print areas including perimeter, infill and support. You can select infill pattern and density before previewing the layers.
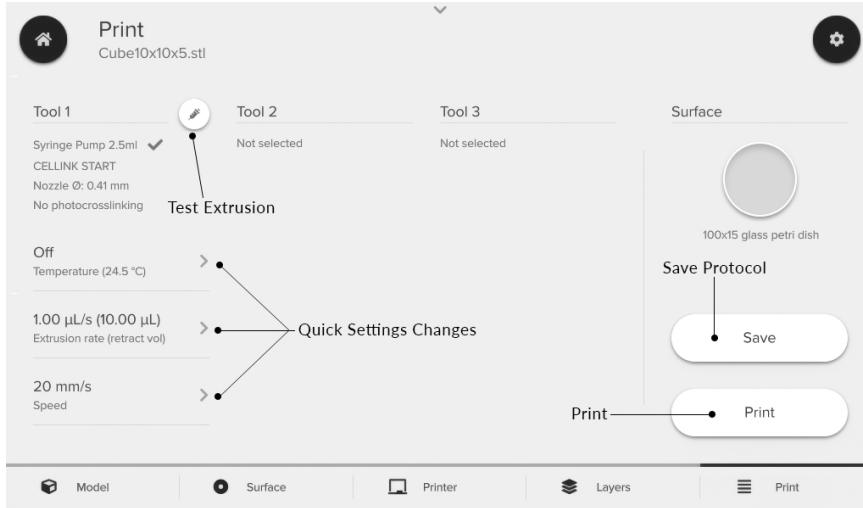
Figure 14: Print menu. You can use this menu to preview print parameters and test the extrusion rate. Parameters can also be adjusted on this menu. Press Print to proceed to the calibration page and press Save to save the configuration as a protocol.

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NOTE: The system will retract the syringe to stop flowing the bioink. This is to ensure the bioink flow is stopped and doesn't continue as a result of trapped air being compressed in the syringe. If the bioink continues to drip, increase the retract volume on the print setup page and retest the bioink flow.

NOTE: Test Bioink Flow must be performed before proceeding to the calibration page. After starting the print, the first system motion will be to revert that retraction volume. If changes are made to the parameters, the bioink must be primed again to ensure the proper parameters are stored.

12. Select Calibrate to calibrate the system (Figure 15).

NOTE: The Syringe Printhead is not compatible with autocalibration.

13. Start the bioprinting process (Figure 15).

Figure 15: Bioprint menu. The system can be calibrated and you can start bioprinting.

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4.3 Removing the Syringe Printhead

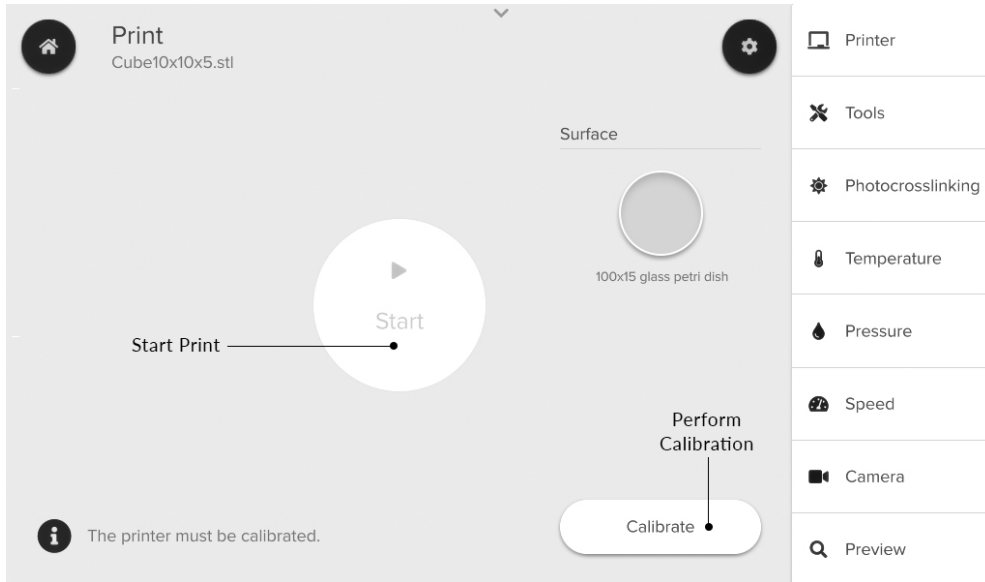1. Move the printhead to the active or loading position using the Tools submenu under the Utilities menu (Section 4.1).
2. Push the syringe plunger lock to the back.
3. Loosen the screw and remove the syringe from the top. Cap your syringe and store it.
4. To remove the printhead, stabilize the top surface of the printhead mount with one hand. Push the printhead upward with the other hand.

4.4 Syringe Printhead optimization

The Syringe Printhead uses a mechanically driven extrusion mechanism which differs from the pneumatic-driven mechanism of standard BIO X printheads. The mechanically driven mechanism requires a different approach to optimize the resulting filament diameter and cell viability. Compared to pneumatic-based extrusion, the Syringe Printhead offers more control over bioink flow rate and dispensed volume. However, this comes at the expense of control over the pressure inside the cartridge and bioink and, by extension, the shear forces placed on cells while printing. Resulting diameters may be easier to control but it may be more difficult to optimize cell viability; the opposite is true of pneumatic printheads.

The theoretical resulting diameter of a filament extruded from the Syringe Printhead is a function of the nozzle diameter, extrusion rate and translation speed of the printhead. The balance between these three parameters will affect the resulting filament diameter. For example, a faster extrusion rate combined with a relatively slower translation rate

will result in a larger diameter. On the other hand, using an extrusion rate that is slow in comparison to the translation speed will result in noncontinuous and nonuniform filaments. In balanced conditions, the resulting diameter will approach the diameter of the nozzle or needle being used. It's important to use the right retraction volume to ensure no excess droplets of material are left behind at starting and stopping points in the printing process, and to ensure continuous filaments. The layer height must also be considered; a layer height that is shorter than the nozzle/needle diameter will result in compressed filaments, while a layer height that is too large will result in poor filament attachment to the substrate due to a discrepancy between the extruded filament and the layer height. A layer height that matches the nozzle/needle diameter might not always be ideal because the bioink can expand or retract after extrusion, or retract as it gels or self-assembles. Layer height can be bioink dependent, so this parameter must be evaluated for each bioink to optimize parameters.

To optimize cell viability, deposit droplets using a set needle diameter and different extrusion rates. Analyzing the resulting droplets after crosslinking and cell culture will enable quantification of the survival rate under the different extrusion rates and shear forces. Then, the optimal extrusion rate can be identified with the necessary translation speed as described above to achieve the target filament diameter.

05

Relevant G-code commands

05 Relevant G-code commands

See the G-code list below to manually code the Syringe Printhead and customize functionality.

Commands	Description
G1 Xnnn Ynnn Ennn Fnnn	When used in combination with the G90 command, which defines absolute coordinates, G1 is the absolute move command. The values for the X and Y parameters are the coordinates (in mm) directing where to move. E tells the BIO X to open the valve for extrusion. F is the movement speed of the printhead in mm/min.
G1 Znnn Ennn Fnnn	When used in combination with the G90 command, which defines absolute coordinates, G1 is the absolute move command. The value for the Z parameter is the coordinate (in mm) directing where to move. E tells the BIO X to open the valve for extrusion. F is the movement speed of the printhead in mm/min.
G4 Snnn Pnnn	The G4 command tells the system to dwell. S is the wait time in seconds and P is the wait time in milliseconds.
G7 Xnnn Ynnn Ennn Fnnn	When used in combination with the G90 command, which defines absolute coordinates, G7 is the relative move command. The values for the X and Y parameters are the coordinates (in mm) directing where to move relative to the current position. E tells the BIO X to open the valve for extrusion. F is the movement speed of the printhead in mm/min.

G7 Znnn Ennn Fnnn	When used in combination with the G90 command, which defines absolute coordinates, G7 is the relative move command. The value for the Z parameter is the coordinate (in mm) directing where to move relative to the current position. E tells the BIO X to open the valve for extrusion. F is the movement speed of the printhead in mm/min.
G92 Xnnn Ynnn Znnn	G92 sets the current position of the printhead to the specified X, Y and Z coordinates. If no values are given, the position is assumed to be 0, 0, 0 (this will also change the Z position).
Tx	Switch between printhead (x). 0, 1 and 2 designate printheads 1, 2 and 3.
M771 TX PYY	M771 turns on the heater in printhead Tx at temperature Pyy. yy is a temperature between 30 and 65 degrees Celsius.
M2045 Tx E	Set syringe direction to extrude; Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2).
M2045 Tx R	Set syringe direction to retract; Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2).
M2032 Tx Sy	Set printhead extrusion rate; Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2) and Sy is extrusion rate in nanoliters/sec.
M2047 Tx E	Fully extrude printhead; Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2).
M2047 Tx R	Fully retract printhead; Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2).
M2051 Tx Vx	Extrude a certain volume; Vx is the volume in nanoliters and Tx is the printhead number (Printhead 1 = T0, 2 = T1, 3 = T2).

06

Frequently asked questions

06 Frequently asked questions

● **Why is the maximum volume in the syringe 2.3 mL?**
The Syringe Printhead is designed for a syringe diameter that corresponds to a specific syringe barrel length. Retracting the syringe beyond this length may cause it to contact the top of the bioprinter chamber and result in damage to the system.

● **What happens if I use a smaller diameter syringe?**
We recommend using the provided syringes or one with an equivalent diameter. The system does not support smaller diameters because smaller diameters are unstable in the Syringe Printhead. The system uses the recommended syringe diameter to calculate the extrusion volume; using a smaller diameter will result in a different extrusion rate.

● **The printhead hits the door during the calibration.**
The Syringe Printhead is larger than other printheads due to the motor and the locking arm. To ensure the printhead does not hit the door during calibration, make sure you select Home to back in the Utilities menu. See Section 4.1 for more details.

● **Why can't I print on the whole build plate?**
The Syringe Printhead is larger than other printheads due to the motor and the locking arm. Due to its size, the printhead cannot reach the back of the build volume. We recommend printing within well plates to print into the front rows of the well plate, then rotating the well plate and resetting the print to print in the remaining rows. You can select the well plates in HeartOS 1.7.

● **What purpose does the retract volume serve?**
The retract volume ensures that bioink extrusion does not continue after the motor stops. Plastic syringe plungers and arms compress during extrusion; this force builds up in the syringe due to bioink compression and small nozzle diameters that impede low-resistance flow. The syringe plunger must be retracted to relieve this pressure. Unlike a pneumatic printhead where air pressure falls and is relieved when the regulator is closed, the Syringe Printhead uses the retract volume to reverse the syringe plunger movement and relieve pressure. Retract volume varies by the bioink, extrusion rate and printing nozzle being used.

● **The beginning of my filament is wider than the end.**
The retract volume may be too large. Before the printhead starts to move, the plunger is compressed to start the flow; this is equal to the retract volume. A retract volume that is too high will extrude and drag the material until the flow rate equals the set extrusion rate, resulting in changing filament diameters. An opposite result implies that the retraction volume is too small.

● **My bioink continues to drip from the syringe after the bioink flow is tested under the utility menu or during the printing process.**
The retract volume may be too small, causing the flow to continue until the pressure is relieved.

06 Frequently asked questions

● **The actual extrusion rate is not matching the set extrusion rate.**
The used nozzle/needle may be too small in diameter or too long for the set extrusion rate or speed. The syringe plunger compression may not be able to compensate for high fluid flow resistance in the nozzle/needle. This might occur more often with high-viscosity bioinks. Use a larger diameter nozzle/needle.

● **My bioink is slipping above the plunger.**
The plunger might be getting worn due to heavy use, or the nozzle might be clogged. Replace the syringe or nozzle.

● **My bioink is not extruding.**
The needle/nozzle might be clogged due to the bioink being used or a pause in printing. Replace the needle/nozzle. If your bioink contains aggregates, ensure they are of a sufficient size to pass through the needle/nozzle without clumping and blocking it. If using a thermosensitive bioink it might have gelled in the tip. Consider using the Temperature-controlled Printhead when printing with thermosensitive bioinks.

● **I am using GelMA or a thermosensitive bioink and my extrusion rate is not consistent.**
The bioink might be gelling at the tip, causing an inconsistent flow due to semigelled material being extruded and replaced by warmer material that flows with less resistance. Consider using a nozzle cover to keep the bioink in the nozzle at the same temperature as the bioink in the syringe barrel.

● My filament is not attaching to the surface during the printing process.
Your layer height may be too high, or your print speed may be too fast. These factors can prevent the filament from attaching to the surface.

● My needle is dragging through my printed structure when it moves.
Your layer height may be too small or your print speed may be too slow. These factors can result in thicker filaments that produce a taller-than-expected print, causing the nozzle to contact the previous layer.

07
Maintenance

07 Maintenance

• Regularly clean the printhead with a damp cloth to remove any dust or debris.

Appendix A: Consumables—needles and nozzles

Appendix A: Consumables- needles and nozzles

Table 2: Compatible needles

Part Number	Type	Color	Gauge	Length (inch)
NZ5180505001	Needle	Green	18	0.5
NZ6200255001	Needle	Pink	20	0.25
NZ6200505001	Needle	Pink	20	0.50
NZ5201005001	Needle	Pink	20	1.00
NZ6210255001	Needle	Purple	21	0.25
NZ6210505001	Needle	Purple	21	0.50
NZ5211005001	Needle	Purple	21	1.00
NZ6220255001	Needle	Blue	22	0.25
NZ6220505001	Needle	Blue	22	0.50
NZ5221005001	Needle	Blue	22	1.00

NZ7220505001	Needle	Steel	22	0.50
NZ6230255001	Needle	Orange	23	0.25
NZ6230505001	Needle	Orange	23	0.50
NZ6231005001	Needle	Orange	23	1.00
NZ6250255001	Needle	Red	25	0.25
NZ6250505001	Needle	Red	25	0.50
NZ5251005001	Needle	Red	25	1.00
NZ6270255001	Needle	Clear	27	0.25
NZ6270505001	Needle	Clear	27	0.50
NZ5271005001	Needle	Clear	27	1.00
NZ6300255001	Needle	Lavender	30	0.25
NZ6300505001	Needle	Lavender	30	0.50

Appendix A: Consumables- needles and nozzles

NZ5320255001	Needle	Yellow	32	0.25
NZ5320505001	Needle	Yellow	32	0.50
NZ5340255001	Needle	Green	34	0.25
NZ5340505001	Needle	Green	34	0.50

Table 3: Compatible conical nozzles

Part Number	Type	Color	Gauge	Length (inch)
NZ4180005001	Conical	Green	18	1.25
NZ4200005001	Conical	Pink	20	1.25
NZ3220005002	Conical	Blue	22	1.25
NZ3250005002	Conical	Red	25	1.25
NZ3270005002	Conical	White	27	1.25

Table 4: Compatible precision conical nozzle

Part Number	Type	Color	Gauge	Length (inch)
NZ2210000801	Micron-s Conical	Light Blue	21	0.75
NZ2230000801	Micron-s Conical	Purple	23	0.75
NZ2250000801	Micron-s Conical	White	25	0.75
NZ2270000801	Micron-s Conical	Red	27	0.75
NZ2300000801	Micron-s Conical	Black	30	0.75
NZ1150000501	Micron-s Conical	Blue	150 µm	0.75
NZ1100000501	Micron-s Conical	Orange	100 µm	0.75
NZ1050000501	Micron-s Conical	Yellow	50 µm	0.75

Appendix A: Consumables- needles and nozzles

Table 5: Compatible cartridges and cartridge accessories

Part Number	Description	Quantity
D16110021153	BD 3-component 3 mL syringe	1
OH000000010	Female/female Luer lock adapter	10
OH000000050	Female/female Luer lock adapter	50

- Official site: **www.cellink.com**
- Contact: **support@cellink.com**
- Contact: **sales@cellink.com**
- Web store: **www.cellink.com/store**

Store

Sales

Official site

Support