BIO X Temperature-controlled Printhead

User Manual

CELLINK®

Contents

1. Package contents 1
2. Technical specifications 3
3. Safety information 7
   3.1 BIO X system warnings 8
   3.2 Temperature-controlled Printhead warnings 8
4. Getting started 9
   4.1 Unpacking and installation 11
   4.2 Optional: Attaching nozzle/needle insulator 12
   4.3 Raising and lowering the printhead 13
   4.4 Loading the bioink cartridge 15
   4.5 Removing the printhead 18
   4.6 Your first bioprint 19
5. Temperature- controlled Printhead optimization 27
6. Relevant G-code commands 31
7. Frequently asked questions 33
8. Maintenance 37
Appendix A: Consumables 39
Support information 46

01
Package contents

01 Package contents

Item	Part number	Quantity
Temperature-controlled Printhead	000000020346	1
Locking screw	000000020106	1

02

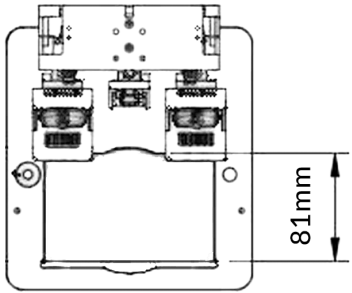
Technical specifications

02 Technical specifications

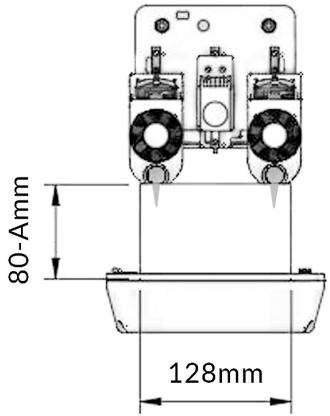
- **Compatible with 3-mL plastic cartridge.**
- **Dimensions (height x width x depth):** 90 x 46 x 58 mm.
- **Weight:** 183 g.
- **Maximum pressure:** 700 kPa.
- **Material composition:**
  - External surfaces: Aluminum.
  - Internal surfaces: Aluminum.
- **Recommended operating temperature:** 20-23 degrees Celsius.

NOTE: We recommend operating BIO X in temperatures between 20 and 23 degrees. In normal operating conditions, the chamber temperature of the BIO X will rise 2-3 degrees above the ambient temperature.

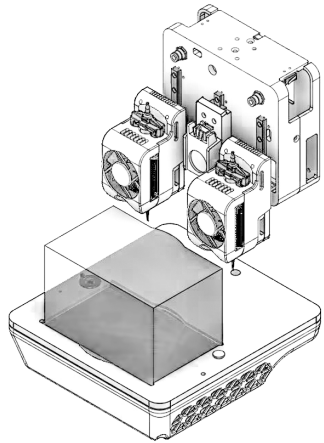
- **Running temperature (in recommended conditions):** 26-32 degrees.
- **Heating capacity and rate:**
  - Total heating range: 65 degrees.
  - Heating rate: Room temperature-65 degrees (10 minutes).
- **Cooling capacity and rate:**
  - Minimum temperature: 17 degrees below chamber temperature.
  - Cooling rate: Room temperature-Δ17 degrees (15 minutes).
- **Build volume (X, Y, Z):** 128 x 85 x (80–A) mm; A represents tip length.



Top View



Front View



Isometric View

Figure 1: Theoretical maximum build volume shown from the top and side view when using the Temperature-controlled Printhead. Exact build volume depends on the cartridge's position in the printhead and the nozzle/needle used. Build volume may be lower when combining the Temperature-controlled 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 common tip lengths. Actual volume may vary based on build plate thickness, size, shape, level, cartridge position and nozzle tightness. 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	9.36 cm	4.8 cm	570 cm^3
Blunt	2.4 cm	0.635 cm	12.7 cm	9.36 cm	5.6 cm	665 cm^3
Blunt	3.0 cm	1.27 cm	12.7 cm	9.36 cm	5.00 cm	594 cm^3
Blunt	4.3 cm	2.54 cm	12.7 cm	9.36 cm	3.70 cm	440 cm^3
Micron-S	1.9 cm	1.9 cm	12.7 cm	9.36 cm	6.12 cm	727 cm^3

03

Safety information

03 Safety information

3.1 BIO X system warnings

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

3.2 Temperature-controlled Printhead warnings

• **DO NOT** clean the printhead by submerging it in liquid or using excessive spraying. Liquid inside the printhead can damage 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 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.
• The Temperature-controlled Printhead can be hot to the touch after use. DO NOT touch the foils when the printhead is heating or cooling.
• **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 EMD Printhead onto the BIO X printhead mounts. Instructions are outlined in the Getting Started section. While loaded on the BIO X, do not pull or push the printhead with excessive force, especially when the BIO X system is turned on. Doing so will damage the motor and its guides.
• **DO NOT** use a jerking motion to remove the printhead. Using a jerking motion risks hitting and damaging the HEPA filter.
• **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.
• The cooling fan of the Temperature-controlled Printhead is exposed to allow for heat dissipation. **DO NOT** put fingers or other objects into the fan while the printhead is in operation. Doing so risks injury and damage to the printhead.
cooling.

04

Getting started

04 Getting started

NOTE: The printing parameters displayed on this manual might not be accurate for your specific protocol. For recommended parameters please consult the documentation for the bioink being used.

1. Cartidge Adapter

2. Cartidge

3. Printhead Body

4. Fan

5. Heat Sink

6. Locking Screw

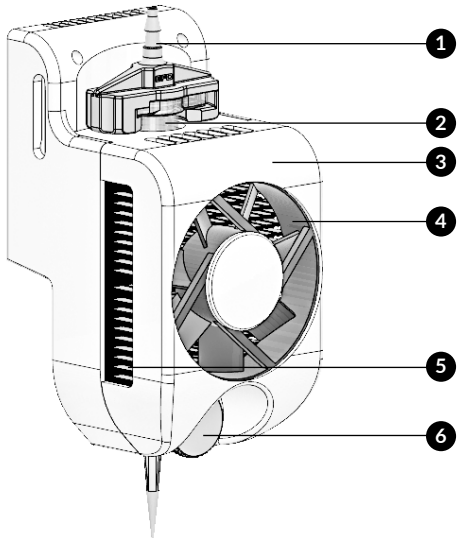


Figure 2: Temperature-controlled Printhead components.

Unpacking and installation

- Open the package. Remove the Temperature-controlled printhead.
- Check for locking screw **(Figure 2)**.
- Check the fan and heatsink for any debris.
- Optional: Attach needle/nozzle insulator:
  - Ensure that the nozzle/needle insulator has the right gauge for the diameter of the nozzle/needle being used.
  - Align the insulator, screws and nozzle **(Figure 3A)**.
  - Insert the collar of the insulator into the Temperature-controlled Printhead.
  - Tighten the screws to secure the insulator to the printhead **(Figure 3B)**.

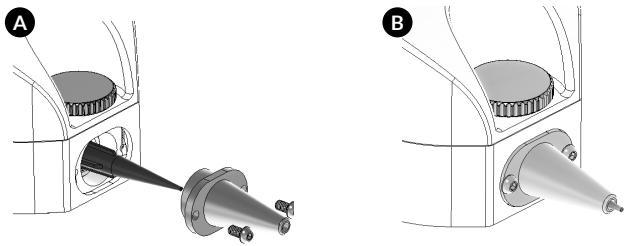


Figure 3: Attaching the nozzle/needle insulator to the Temperature-controlled Printhead.

04 Getting started

• To install the printhead, stabilize the bottom of the printhead mount with one hand to avoid straining the motor. Align the printhead above the desired printhead mount and push downward using your opposite hand **(Figure 4)**.

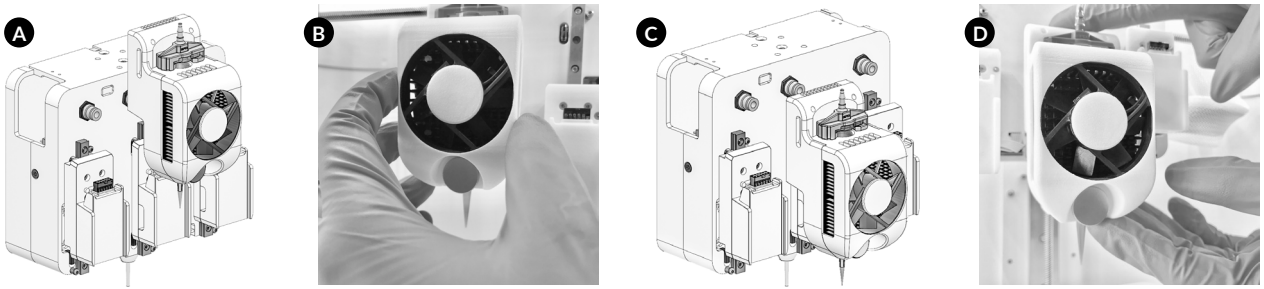


Figure 4: Inserting the Temperature-controlled Printhead into the BIO X system.

Raising and lowering the printhead

- On the user interface, navigate to the Utilities menu and then go to the Tools submenu.
- Lower the desired printhead mount into the active or loading position by pressing the down arrow **(Figure 5)**.
- Press the up arrow to return the printhead to the nonactive position.

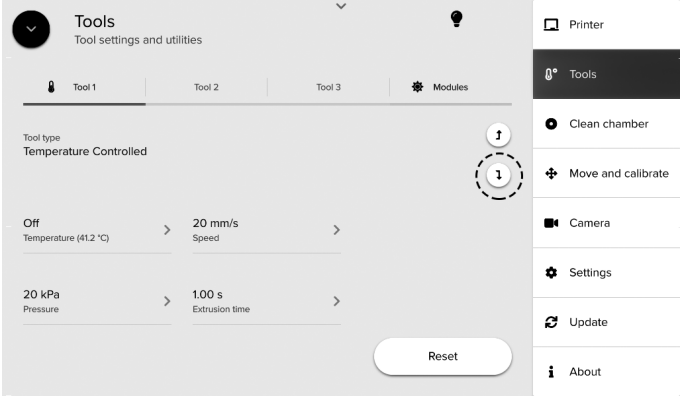


Figure 5: Lowering the printhead into the active position.

04 Getting started

Preheating or precooling the Temperature-controlled Printhead

• Set a temperature in the BIO X software using the **Utilities** menu and **Tools** submenu. When cooling, the operating temperature range of the Temperature-controlled Printhead has a maximum of 17 degrees below the chamber temperature; when heating, the maximum temperature is 65 degrees **(Figure 6)**.
• Wait for the the Temperature-controlled Printhead to reach the set temperature.

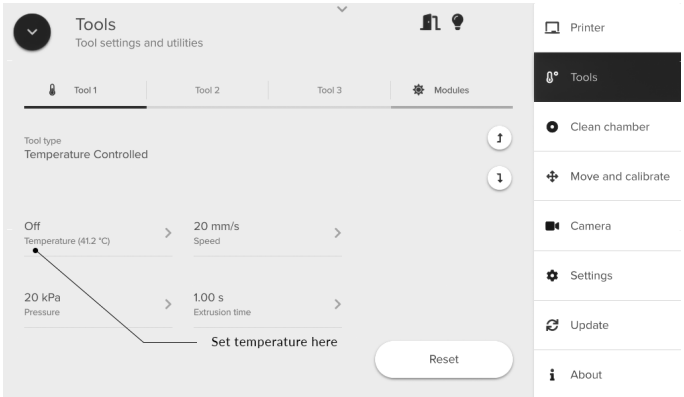


Figure 6: Preset temperature for the Temperature-controlled Printhead.

NOTE: We recommend precooling or preheating your bioink in the cartridge outside the printhead in an incubator, water bath or refrigerator before use. If using a water bath, ensure the cartridge is dry before putting it in the printhead.

Loading the bioink cartridge

- Fill a cartridge with bioink or use a prefilled cartridge.
- Remove the tip cap **(Figure 7A)**. Attach a nozzle **(Figure 7B)**. Remove the end cap **(Figure 7C)**.

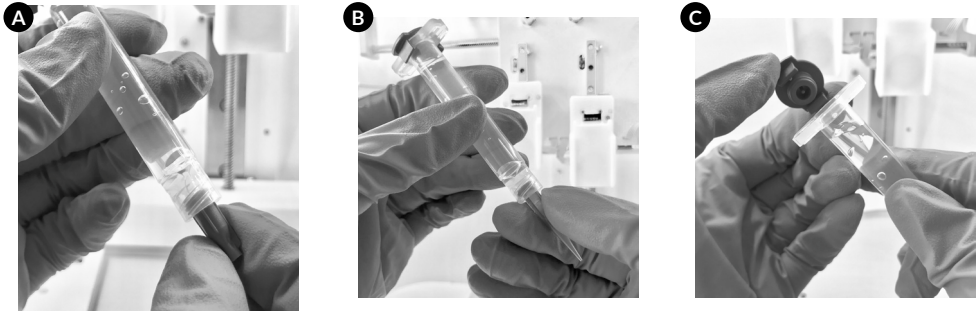


Figure 7: Preparing a prefilled bioink cartridge.

• Align the cartridge adapter to the loaded cartridge **(Figure 8A)**. Twist until secure **(Figure 8B)**.

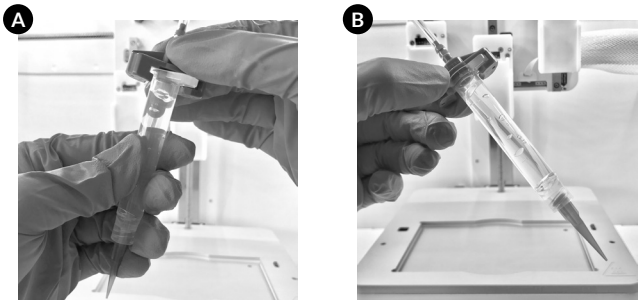


Figure 8: Attaching the air adapter to the cartridge.

• Load the cartridge into the printhead from the top **(Figure 9B)**. Tighten the locking screw. To make this step easier, we recommend placing the printhead in the active position (lowered) as explained in the Raising and lowering the printhead section (page 13) **(Figure 9A)**.

• Connect the air tubing to the air inlet on the printbox above the respective printhead **(Figure 9C)**.

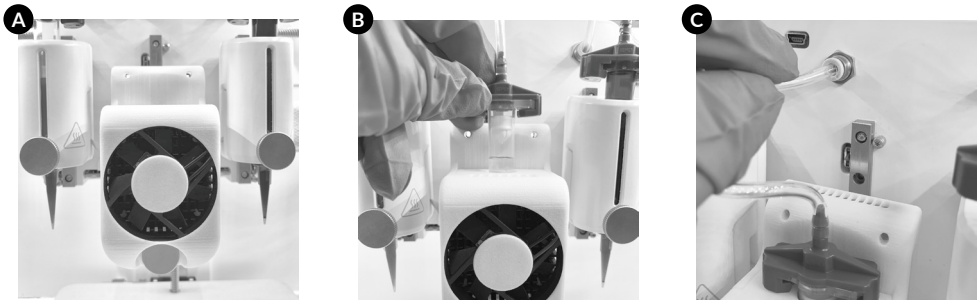


Figure 9: Inserting the cartridge into the Temperature-controlled Printhead.

04 Getting started

Removing the Temperature-controlled Printhead

• To remove the printhead, use one hand to stabilize the printhead mount and the other hand to push the printhead slightly upward. **(Figure 10A)**.
• Hold the printhead with one hand and detach the tubing from the printbox with the other hand **(Figure 10B)**.

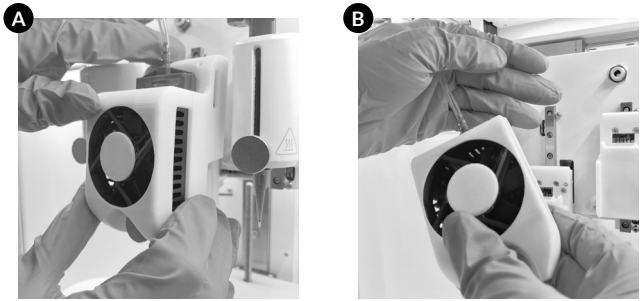


Figure 10: Removing the Temperature-controlled Printhead from the BIO X printhead mount.

Your first bioprint

- Prepare the bioink cartridge and preheat or precool the printhead **(page 12-17)**.
- Select Bioprint from the Start menu **(Figure 11)**.

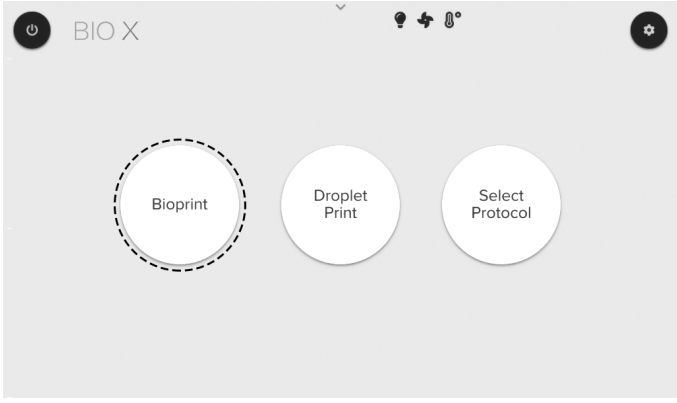


Figure 11: The Start menu. Select Bioprint to set up a print.

• Select an STL file (filename.stl) or G-code file (filename.gcode) from the Model menu (Figure 12). Proceed to the next menu by selecting Surface along the bottom of the screen.

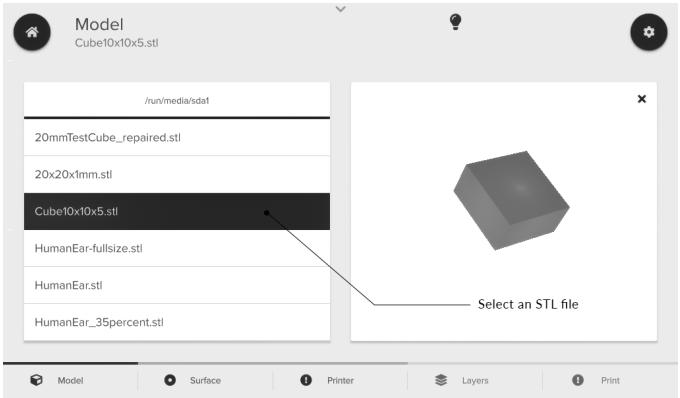


Figure 12: Selecting an STL file from the Model menu.

- Select the surface to print on and proceed to the next menu **(Figure 13)**.

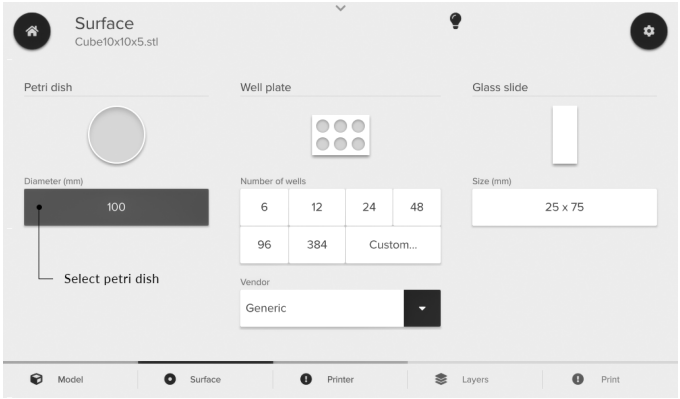


Figure 13: Select print surface from the Surface menu.

• Select the tool position where the printhead has been attached. Ensure that Temperature-controlled is selected under the tool type **(Figure 14)**.

• Enter the desired printing parameters for the printhead **(Figure 14)**. Proceed to the Layers menu when finished. Parameters necessary for the pneumatic printhead include:

1) Nozzle diameter.
2) Pressure.
3) Print speed.
4) Preflow delay.
5) Postflow delay.

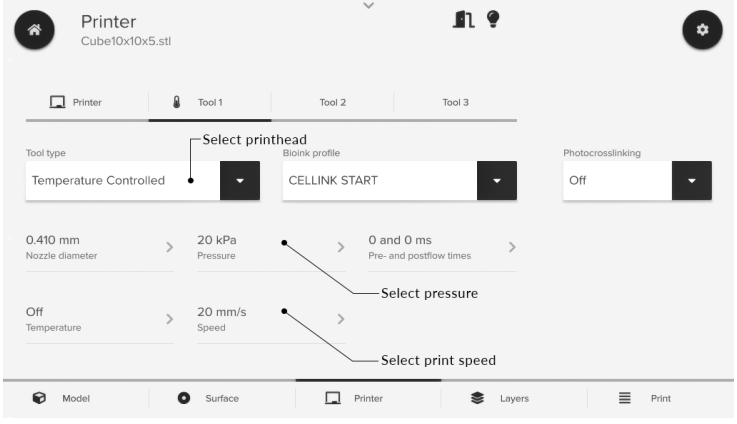


Figure 14: Printing parameters to set on the Printer **menu.**

• Assign the enabled printhead to the respective layer characteristics. Proceed to the next menu **(Figure 15)**.

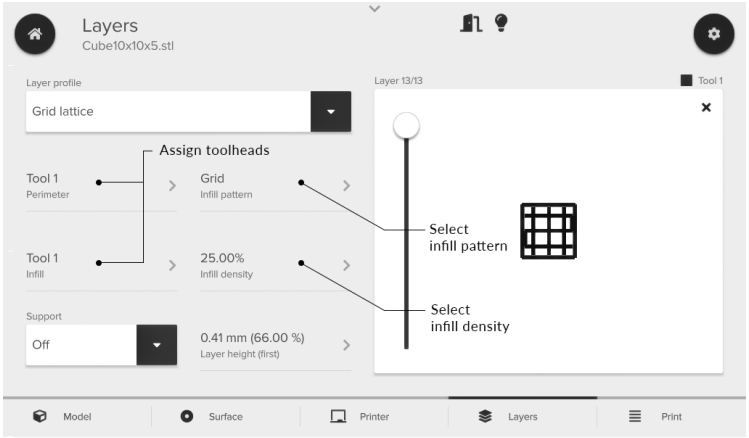


Figure 15: Layer menu. Toolheads can be assigned to respective print areas like perimeter, infill and support. You can select infill pattern and infill density and preview the layers.

• Prime the nozzle/needle. Use the Drop button next to the toolhead number to test bioink flow **(Figure 16)**.

Press Print to proceed to the calibration page.

Figure 16: Use the Print menu to preview print parameters and test the extrusion rate. You can also adjust print parameters on this menu. Press Print to proceed to the Calibration menu and press Save to save the configuration as a protocol.

- Select Calibrate to calibrate the system to the desired start position **(Figure 17)**.
- Start the bioprinting process **(Figure 17)**.

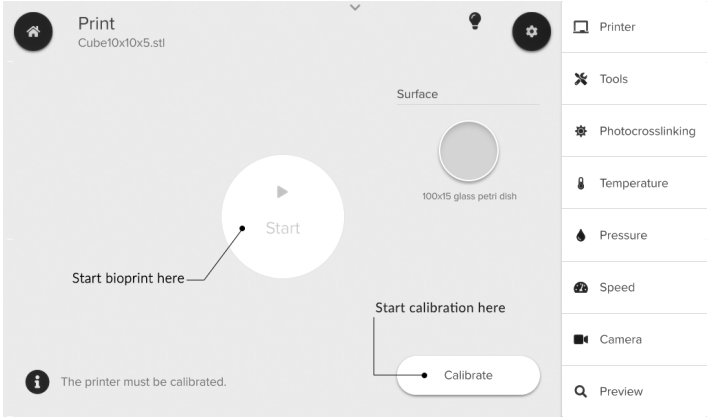


Figure 17: Use the Bioprint menu to calibrate the system and start the bioprinting process.

05

Temperature-controlled Printhead optimization

05 Temperature-controlled Printhead optimization

The Temperature-controlled Printhead uses compressed air to extrude a bioink through a nozzle or needle with a diameter between 50 and 600 micrometers. As a result, you can increase and decrease the air pressure to control the rate at which the bioink filament is dispensed. Bioinks should be thoroughly characterized to determine the best parameters to use with the Temperature-controlled Printhead.

Applied pressure must be controlled to maintain viability while bioprinting. Excessive shear stress resulting from high pressures or small nozzle diameters can cause cells to damage or rupture. Pneumatic-based extrusion systems offer better control over cartridge pressure and the resulting cell viability than mechanical-based extrusion systems. However, pressure does have a direct relationship to flow rate and the resulting filament diameter.

The theoretical diameter of a filament extruded from the Temperature-controlled Printhead is a function of the pneumatic pressure, nozzle diameter, translation speed, layer height, bioink chemistry and temperature.

- As the applied pressure increases, the filament diameter will increase.
- As the translation speed increases, the filament diameter will decrease. Toward the extreme parameters, filaments will be noncontinuous and nonuniform.
- In balanced conditions, the filament diameter will approach the inner diameter of the nozzle or needle used.

05 Temperature-controlled Printhead optimization

Using the Temperature-controlled Printhead to change the viscosity of the bioink by increasing or decreasing the temperature will result in similar changes. To ensure continuous filaments, you can set preflow delays at the beginning of each layer and at various starts and stops. The bioink's mechanical compression in the cartridge can result in a flow delay. The preflow delay setting pauses the printhead movement for the selected time after the air pressure valve is opened. This enables the bioink to start extruding before the printhead starts moving, resulting in more continuous filaments at faster printing speeds.

The layer height also plays a role in your prints. A layer height that is shorter than the nozzle/needle inner diameter will result in a wider filament, while a layer height that is too large will result in poor filament attachment. A layer height that matches the nozzle/needle diameter may not always be ideal because the extruded bioink can expand or contract as it gels or self-assembles. This parameter is bioink- and nozzle-dependent, and must be evaluated for each bioink.

Use the droplet printing menu to deposit droplets. To optimize cell viability, we recommend depositing cells in droplets using a pressure gradient. Analyze the resulting droplets after crosslinking and cell culture to quantify the survival rate and determine acceptable cell loss for different pressure settings. The optimal pressure can be matched with the translation rate and nozzle necessary to achieve the target filament diameter.

05 Temperature-controlled Printhead optimization

To enhance temperature control at the tip and prevent clogging, we recommend using a nozzle/needle insulator compatible with the nozzle or needle used for your protocol.

See the G-code Section to program the Temperature-controlled Printhead and enable more customized functionality.

06

Relevant G-code commands

06 Relevant G-code commands

Table 2: Relevant G-code commands.

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 of 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. The F parameter is the 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 indicates the coordinate (in mm) directing where to move. E tells the BIO X to open the valve for extrusion. The F parameter is the speed of the printhead in mm/min.
G4 Snnn Pnnn	The G4 command instructs the system to dwell. The S command 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. The F parameter is the 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 indicates the coordinate (in mm) directing where to move relative to the current position. E tells the BIO X to open the valve for extrusion. The F parameter is the speed of the printhead in mm/min.

06 Relevant G-code commands

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	Tx switches to printhead (x), where 0, 1 and 2 designate printheads 1, 2 and 3.
M771 Tx Pyy	M771 turns on the heater in printhead Tx at temperature Pyy, where yy is a temperature between 30 and 65 degrees Celsius.
M750 Tx Py Dz	Extrude from printhead x at pressure y for z milliseconds.
M751 Tx	Stop extrusion from printhead x.

07

Frequently asked questions

07 Frequently asked questions

• **The Temperature-controlled Printhead is not reaching the set temperature.**
Make sure that your set temperature is within the range of the Temperature-controlled Printhead specifications (between 17 degrees Celsius below the chamber temperature and 65 degrees Celsius). Ensure the main fan runs when the printhead is changing temperature. If it isn't, blow air straight at the fan to check if you can manually rotate it. If the fan doesn't rotate, check if debris is preventing the fan from running. If it does rotate while blowing air at the fan but not when regulating temperature, contact support@cellink.com.

• **The printhead hits the door when calibrating.**
The Temperature-controlled Printhead is larger than other printheads due to its fan and heat sink. Ensure Calibrate to the back is selected in the Utilities menu.

• **Can I use multiple printhead types simultaneously?**
You can use multiple printheads within the same printing protocol to generate multimaterial constructs. However, you cannot print simultaneously as the pressure can only be provided to one printhead at a time.

• **What is the mechanism that enables bioprinting with this printhead?**
The Temperature-controlled Printhead leverages pneumatic microextrusion bioprinting. Compressed air actuates a plunger by opening and closing a valve, causing bioink in the cartridge to extrude.

07 Frequently asked questions

• My bioink is not extruding.
The needle/nozzle may be clogged. Certain bioinks are more likely to clog. A pause in use can also cause clogging. Replace the needle/nozzle and make sure that your bioink's aggregates are small enough to pass through the needle/nozzle. Use a nozzle/needle insulator to ensure heat dissipates to the nozzle or needle.

• I am using GelMA or a thermosensitive bioink and my extrusion rate is not consistent.
The inconsistent flow may be due to semi-gelled material being extruded and replaced by the warmer material that flows with less resistance. Try using a nozzle cover to keep the bioink in the nozzle at the same temperature as the bioink in the cartridge.

• 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.

• My needle is dragging through my printed structure while moving.
Your layer height may be too small or your print speed may be too slow. These parameters can result in thicker-than-expected filaments, creating a print that is taller than expected and resulting in the nozzle contacting the previous layer.

08
Maintenance

08 Maintenance

- Store the printhead in a safe place where it will be protected from falls, such as a drawer.

- Clean the printhead regularly with a damp cloth to remove dust and debris.

- If cooling performance is poor, clean the heat sink. While uninstalled, hold the printhead with a finger preventing the main fan from rotating. Blow compressed air into the side vent of the printhead and over the heat sink to remove dust.

Appendix A: Consumables

Appendix A: Consumables

Table 3-1: Compatible needles.

PART NUMBER	TYPE	COLOR	GAUGE	LENGTH (INCHES)
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

Appendix A: Consumables

Table 3-2: Compatible needles.

PART NUMBER	TYPE	COLOR	GAUGE	LENGTH (INCHES)
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

Appendix A: Consumables

Table 3-3: Compatible needles.

PART NUMBER	TYPE	COLOR	GAUGE	LENGTH (INCHES)
NZ6300255001	Needle	Lavender	30	0.25
NZ6300505001	Needle	Lavender	30	0.50
NZ5320255001	Needle	Yellow	32	0.25
NZ5320505001	Needle	Yellow	32	0.50
NZ5340255001	Needle	Green	34	0.25
NZ5340505001	Needle	Green	34	0.50

Appendix A: Consumables

Table 4: Compatible conical nozzles.

PART NUMBER	TYPE	COLOR	GAUGE	LENGTH (INCHES)
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

Appendix A: Consumables

Table 5: Compatible precision conical nozzles.

PART NUMBER	TYPE	COLOR	GAUGE	LENGTH (INCHES)
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

Table 6: 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
D16110021357	TCPH conical nozzle insulator	1
D16110021358	TCPH steel tip insulator	1

Support information

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

Store

Sales

Official site

Support