BIONOVA X

User Manual

Accelerating Precision Medicine

Contents

1. Safety and compliance

Before you start using your BIONOVA X™ bioprinter, please carefully review the entire manual. Improper operations of the bioprinter can lead to personal injury and equipment damage.

1.1 General safety information

Keep your workplace tidy. Do not operate the BIONOVA X™ bioprinter in the presence of flammable liquids, gases, or dust. The BIONOVA X™ bioprinter should be operated by the trained personnel, who are aware of the risks and dangers that can be induced to themselves and the staff in close proximity to the bioprinter. The staff involved in the installation or maintenance of the system, or a part of the system, must be qualified through appropriate training. Use the instrument only for its intended purpose as described in the documentation. Do not modify the instrument, sub-components, or accessories.

WARNING!

- Please do not refit the BIONOVA X™ bioprinter plug.
- Please do not use the BIONOVA X™ bioprinter in damp or wet environment.
- Please unplug the BIONOVA X™ printer if you do not intend to use it for a long period of time to prevent uncertain accidents.
- Never reach into the bioprinter while it is in operation. BIONOVA X™ printer has many different moving parts that can cause personal injuries.
- Do not leave the printer unattended while it is in operation.
- Always wear protective gear including gloves and eye protection when using the printer or handling hazardous materials.
- Before cleaning, inspecting, adjusting, repairing, or disassembling the instrument or the dispensing units, stop the instrument, turn off the main switch, and disconnect it from its electrical power source.
- Verify that all connections are properly connected and all components are properly mounted or installed before using the bioprinter.
- Only use consumables that are designed for and compatible with the BIONOVA X™ bioprinter.

1.2 Protective equipment

WARNING!

- Always wear protective goggles and gloves, and make sure the environment around the instrument is secure from other personnel while operating the printer.
- Always wear protective goggles and gloves while handling dangerous materials that can be absorbed by the skin or which are toxic, strike-attractive, corrosive, allergy inducing, carcinogenic, reproduction-endangering, or mutagenic to humans.
- Always read the safety data sheets, packing labels, and manufacturer's catalogue before use, and make sure to use adequate safety equipment.
- Please keep your hair, clothing, and gloves away from moving parts.

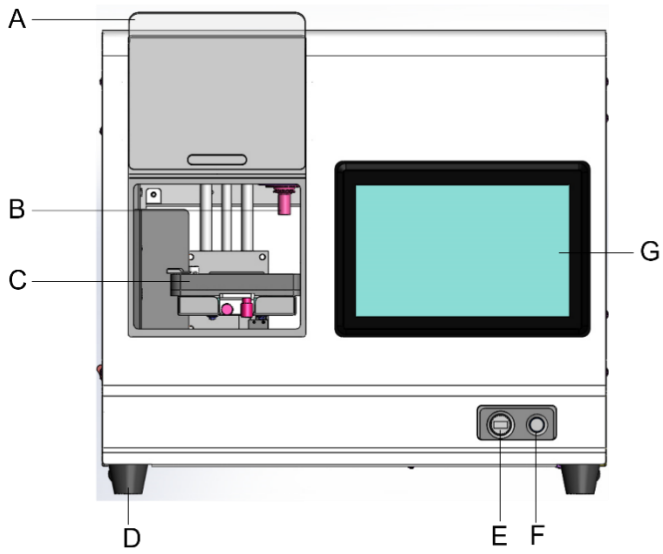
1.3 Opening the product and service

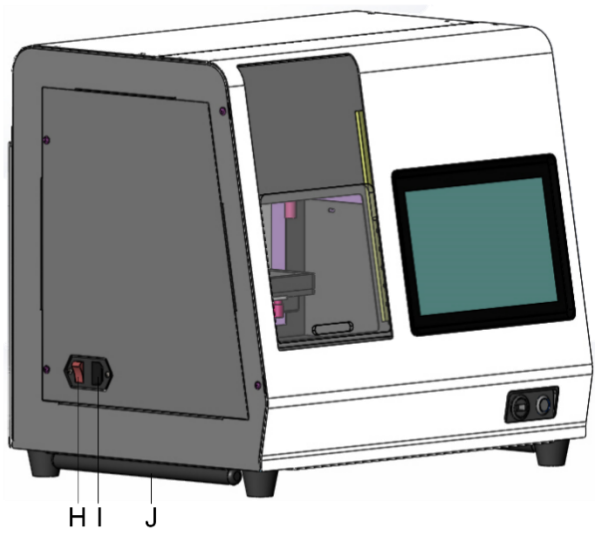
WARNING!

- The BIONOVA X™ **SHOULD NOT** be taken apart. Doing so endangers both the users and the equipment, which will also void the warranty.
- Never use the device for illegal activities.

2. Specifications

2.1. BIONOVA X™ Diagram





A. Sliding door

B. Printing probe

C. Well plate tray

D. Rubber legs

E. USB port

F. Power button for the computer

G. 10” glove friendly touchscreen

H. Power switch for the printer

I. Power supply receptacle

J. Handles for lifting

2.2. Technical Specifications

Bioprinting technology	Digital light projection based stereolithography system
Dimensions	20.3” (W) x 15” (D) x 17.4” (H) 515 mm (W) x 380 mm (D) x 441 mm (H)
Weight	90 lbs (41 kg)
Resolution	10 µm
LED wavelength	405 nm

Heater temperature	Room temperature to 60 °C
Well plate format	24 well plate, 12 well plate, 6 well plate
Support file types	.stl, .png, .bmp, .jpg
Connectivity	1x USB port
Display	10” touch screen, glove friendly
Power supply input	100-240VAC, 50-60Hz, 200W

3. Getting started with BIONOVA X™

3.1. Unpacking and installation

Please refer to the Unpacking Instructions (emailed to the customer separately) for detailed instructions with photos.

1. Remove the screws (six screws in total) located on the four sides of the bottom pallet of the crate.

2. Carefully lift the top part of the crate (i.e., the top panel with four side panels attached to it) and remove the foam around the printer.

3. Lift the printer by grasping the printer firmly from the bottom by two people and set it on a leveled and steady table or biosafety cabinet surface.

4. Open the sliding door and remove the foam padding around the well plate tray.

5. Remove the right-angle support under the well plate tray by unscrewing the two red screws

6. Use the power cord to connect the printer to a power supply via the port located on the left side of the printer.

7. Power on the printer by pushing the power supply switch located on the side of the printer.

8. Power on the computer by pushing the power button located in the front of the printer.

3.2. Contents of the box

Printer related

- 1x BIONOVA X™ printer
- 1x Printer probe adapter
- 1x Hex key for printer probe adapter
- 1x Power cord
- 1x Bluetooth keyboard with touchpad
- 1x USB hub
- 1x User manual

Sample consumables (optional; in separate box)

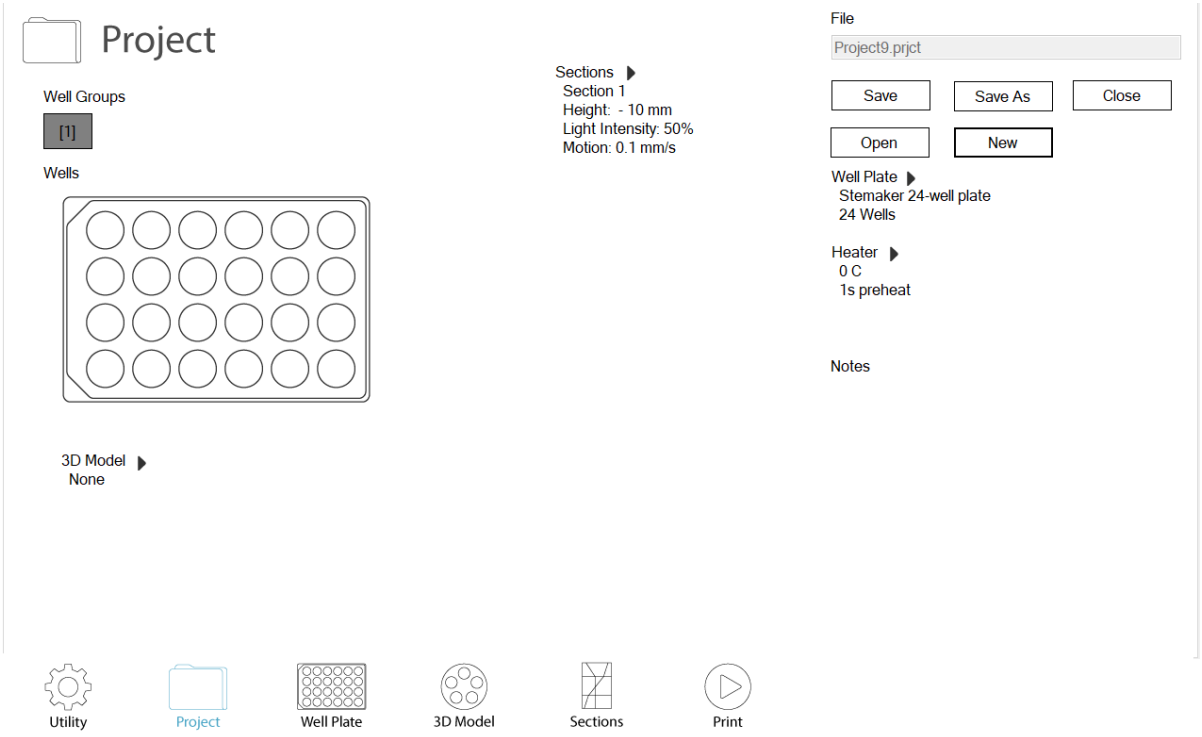
- PEGDA [Poly(ethylene glycol) diacrylate] Sterile Starter Bioink
- Sample Probes for 6-Well Plates
- Sample Probes for 12-Well Plates
- Sample Probes for 24-Well Plates
- Sample 6-Well Plates
- Sample 12-Well Plates
- Sample 24-Well Plates

4. Your first bioprint with BIONOVA X™

4.1. Setting up BIONOVA X™

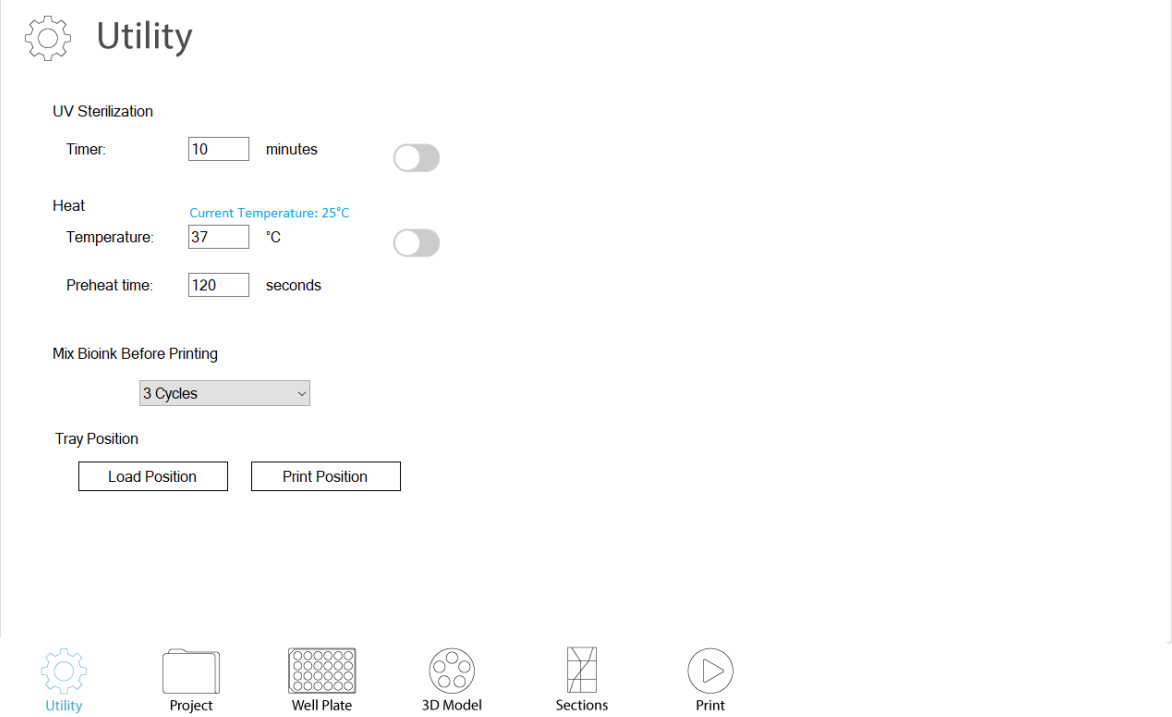
1.. Power on the printer by pushing the power supply button located on the left side of the printer.

2. Power on the computer by pushing the power button located in the front of the printer. The printer will initialize the software and the position of the well-plate tray. After initialization, the software interface will load the blank Project page as shown below.



3. Make sure the sliding door is closed.

4. Go to the **Utility** page and turn on the UV sterilization for the desired time.



4.2. Loading bioprinting probe

1. Once the UV sterilization is completed, open the sliding door.

2. Remove the provided printing probe from the sterile packaging in a biosafety cabinet.

Caution: Choose the right printing probe (i.e., 6, 12, or 24-well probe) to match the well plate (i.e., 6, 12, or 24-well plate) to be used for printing.

3. Carefully retrieve the magnetic probe adapter from above the well-plate tray. Mount the probe to the adapter of the BIONOVA X^{TM} printer by pressing the large end of the probe into the adapter and then mount the adapter with the probe onto the printer. Make sure the probe is mounted properly and tightly.

Tip: Use the provided 1/16-inch hex key to adjust the four screws on the magnetic probe adapter to fit the probes if needed.

Caution: Pay attention to not touch the well-plate tray when inserting or removing the printing probe.

4.3. Preparing and loading bioink

1. Choose the provided well plate to match the loaded printing probe.

2. Remove the well plate from the sterile packaging in a biosafety cabinet.

3. Load the bioink to the well plate and do not exceed the maximum volume suggested in the following table.

4. Press the **Load Position** button on the **Utility** page and carefully load the well plate onto the tray after it is moved to the load position.

Caution: Please make sure the well plate is placed in the right orientation as shown on the **Project** page and secure the well plate in the tray using the clamp.

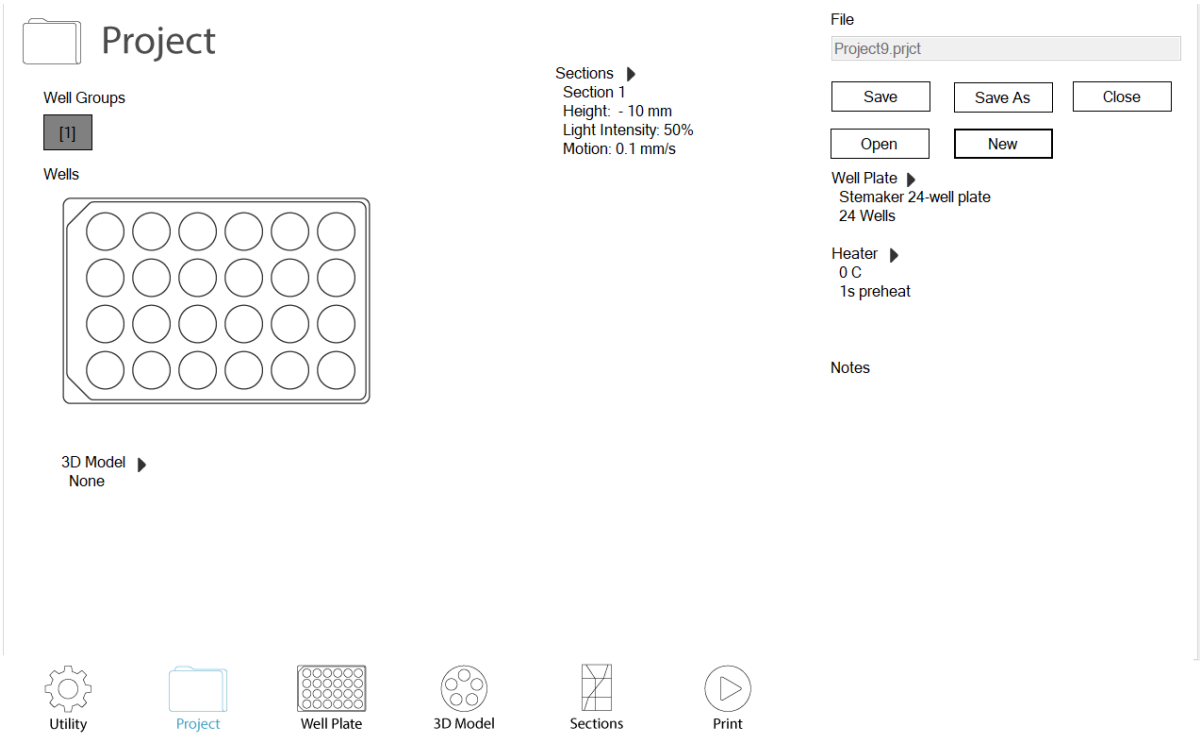
Printing probe and well plate used	6-well probe and 6-well plate	12-well probe and 12-well plate	24-well probe and 24-well plate
Maximum volume of bioink for each well (ml)	8.6	3.4	1.3

4.4. Setting up a bioprinting project

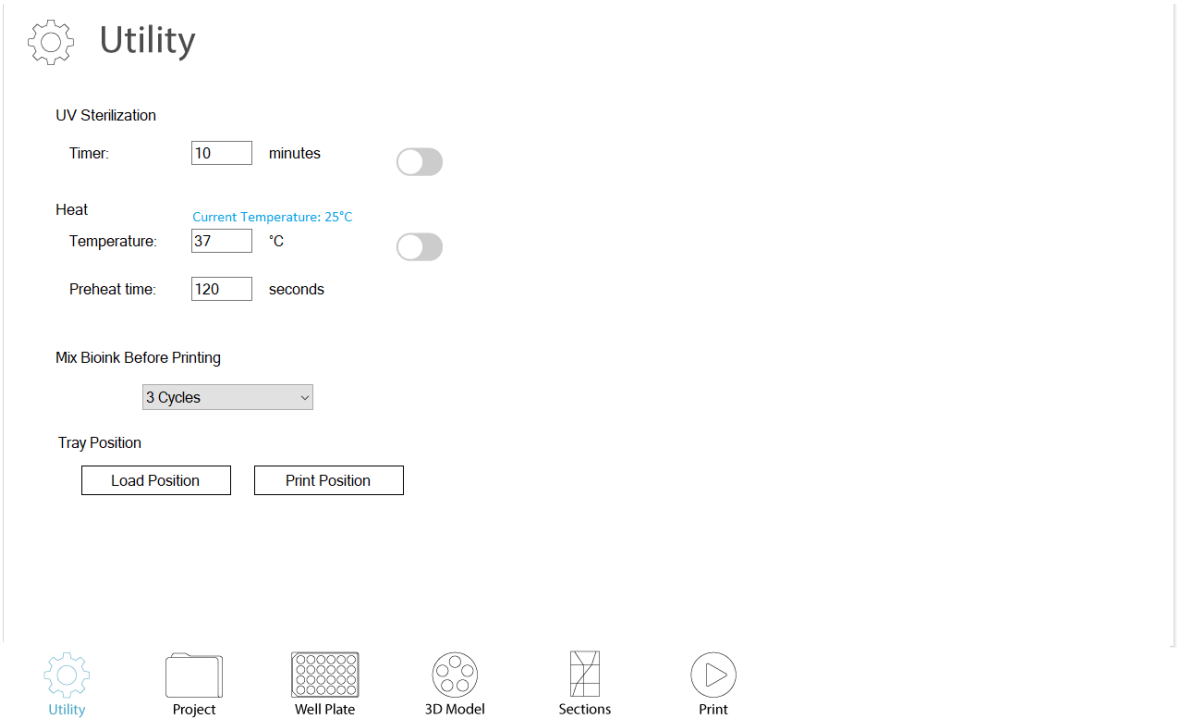
1. Go to the **Project** page and press **New** to start a new printing project. The **Project** page provides an overview of the bioprinting project and settings.

2. Click **Save As** button to input desired Project Name and choose the folder to save the project.

3. You can also **Open** a previously saved project.

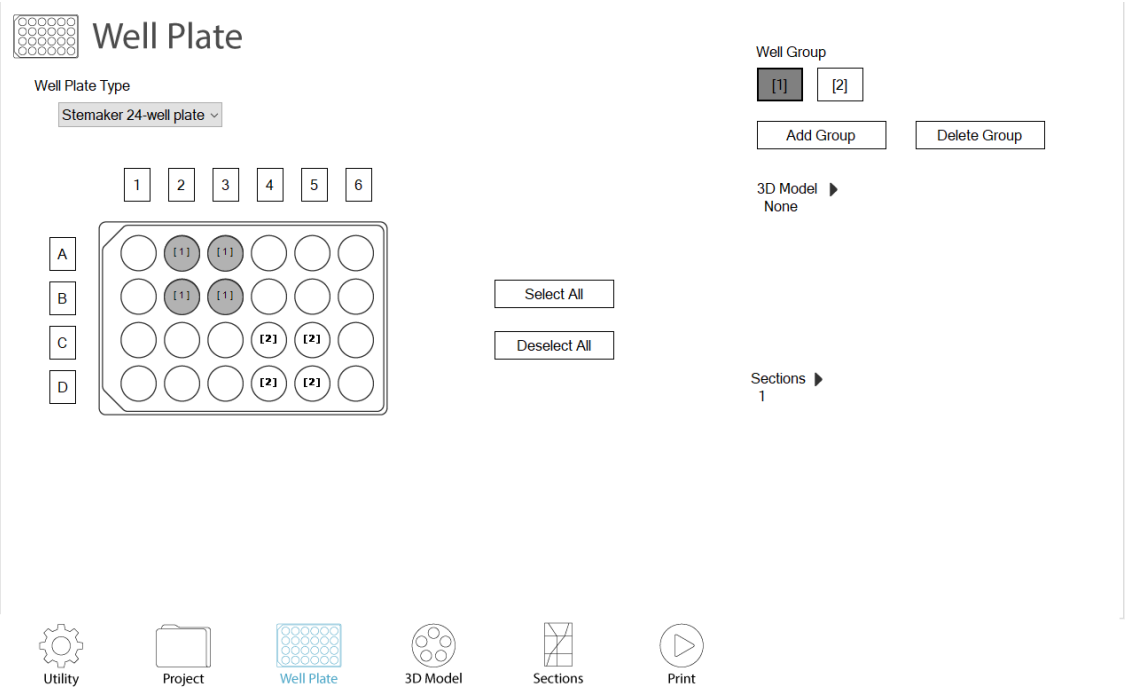


4. If heating or mixing of the bioink is needed, go to the **Utility** page and change the desired settings.

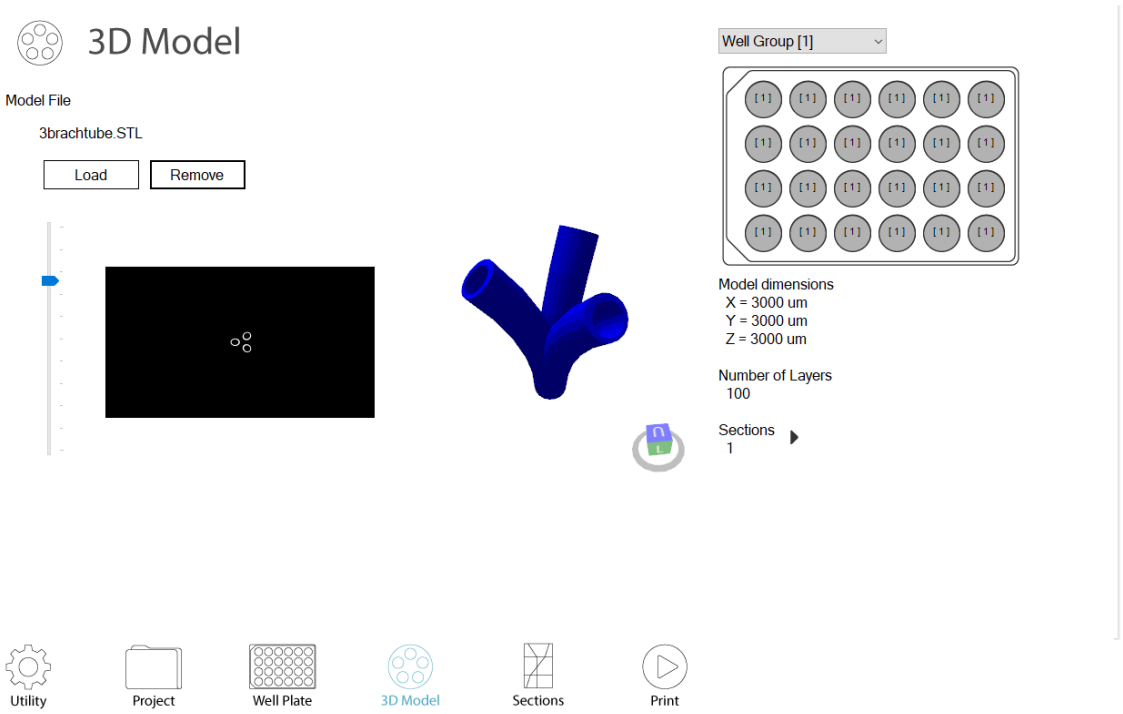


5. Go to the **Well Plate** page and choose the desired well plate type from the drop-down menu.

6. Well groups can be added or deleted. Wells can be assigned to or removed from each well group by selecting or deselecting the wells when the target well group is highlighted on the right panel.

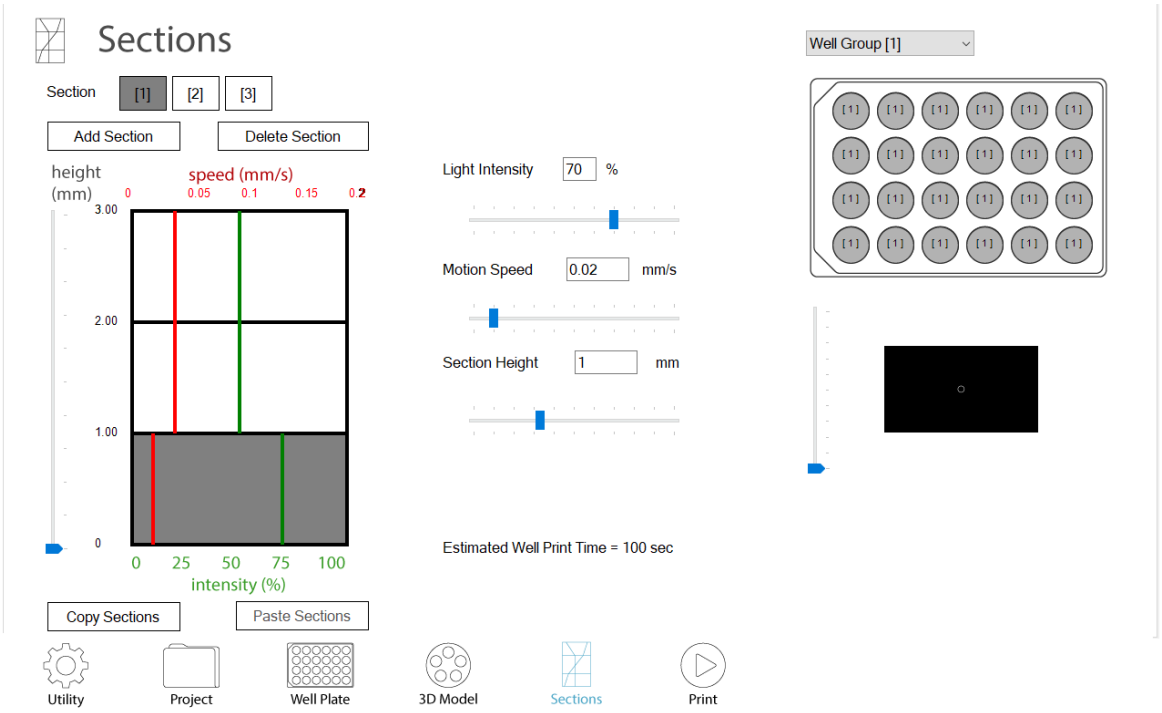


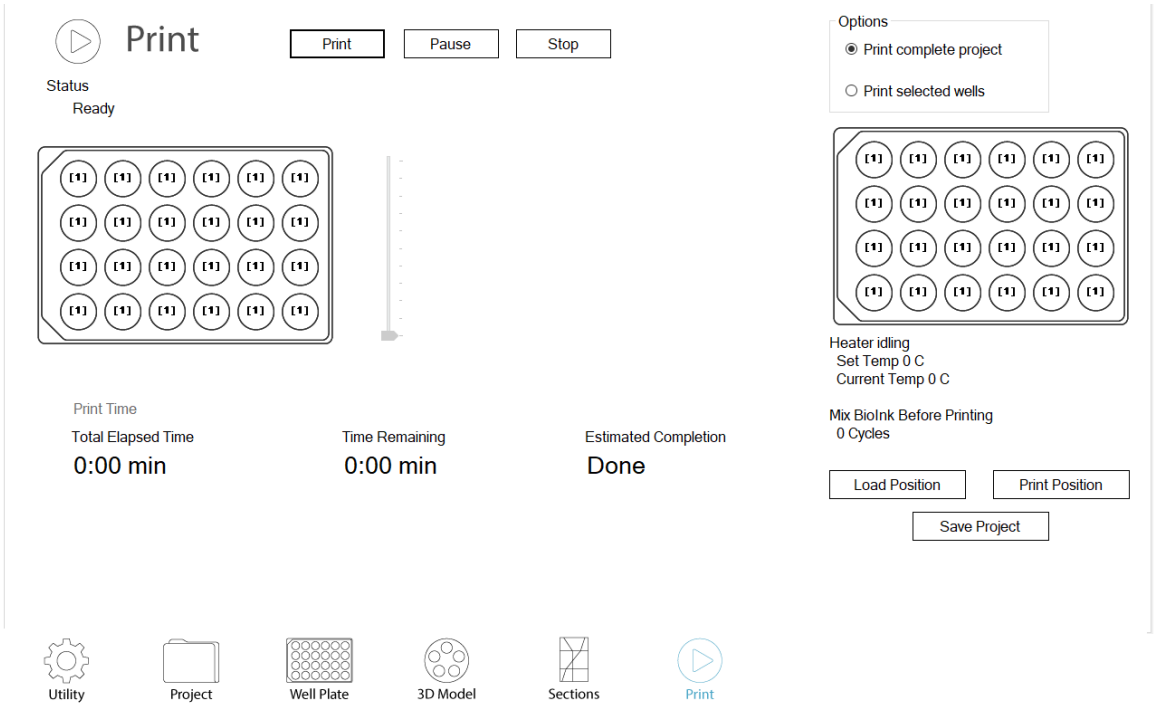
7. 3D models (.stl files) or 2D image files (.png, .jpg, .bmp files) can be loaded for each well group on the **3D Model** page. When loading 3D models, the software will ask for the maximum dimensions in x, y, z directions and the number of layers to be sliced. When loading 2D image files, the software will only ask for the dimension in z direction as the x, y dimensions are set by the 2D image design. The table below provides the maximum dimensions that can be printed in x, y, z directions for each combination of printing probes and well plates.



Printing probe and well plate used	6-well probe and 6-well plate	12-well probe and 12-well plate	24-well probe and 24-well plate
Maximum dimension in X direction (mm)	19.2	9	7
Maximum dimension in Y direction (mm)	10.8	9	7
Maximum dimension in Z direction (mm)	9	9	6.5

8. For each 3D model or 2D image loaded for each well group, you can set one single section or multiple sections with variable printing parameters (i.e., light intensity, motion speed, section height) in the **Sections** page.





9. Once all the parameters are set, you can choose to print the complete project or only selected wells in the **Print** page. Start printing by pushing the **Print** button. The finished wells will be shown as green, the ongoing well will be shown as red, and the selected wells to be printed are shown as grey.

10. You can pause or stop the printing at any time during the printing.

11. Once the printing is stopped or completed, the well plate tray will automatically return to the load position.

4.5. Retrieving the bioprinted samples

1. Please make sure the tray is in the load position before retrieving the well plate or the printing probe.

2. Open the sliding door and carefully remove the well plate from the tray.

3. Gently remove the uncured bioink solution from the wells and gently rinse the bioprinted samples with desired buffer solution or cell culture medium. The bioprinted samples are ready for culturing, imaging or assaying.

4. Carefully retrieve the printing probe without touching the well plate tray and remove it from the magnetic probe adapter. Clean and store the printing probe in a sterile container for later use.

Caution: Printing probes are consumables and they will wear out over time depending on the use. We do not recommend using the same probe for different bioinks or by different users which could lead to potential cross-contamination.

5. Return the magnetic probe adapter to the printing chamber.

6. Close the sliding door.

4.6. Powering off the printer

1. After the printing is finished, turn off the computer by pressing the power button in the front of the printer.

2. Turn off the power supply of the printer by pushing the power switch on the left side of the printer.

Caution: When an emergency stop is needed, push the power switch on the left side of the printer directly to shut down the printer.

5. Consumables

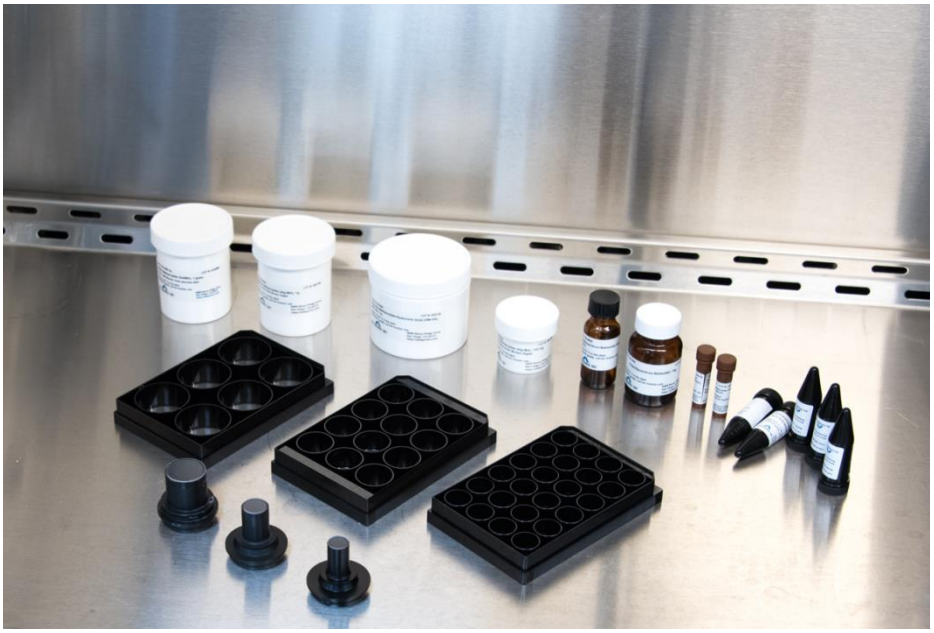
5.1. Bioprinting probes

Our probes are specially designed for our BIONOVA X™ 3D printer to print directly into multi-well plates (24, 12, and 6-well plate). The probe tips are specially treated to prevent adhesion of 3D printed scaffolds to the probe during printing.

Our probes are available in three tip sizes: small, medium and large, which are designed specifically to work with our 24-well plate, 12-well plate and 6-well plate, respectively.

To use the bioprinting probe

- Choose the appropriate bioprinting probe for different multi-well plates. For example, use the probe with a small tip for the 24-well plate, the probe with a medium tip for the 12-well plate, and the probe with a large tip for the 6-well plate.
- The new probe is sterile and comes in a sterile plastic bag. The new probe can be used directly for bioprinting. The probe can be reused. Replace the probe with a new one when there is any visible mark on the tip of the probe.
- To use the probe, mount the probe to the magnetic adapter of the BIONOVA X™ printer by pressing the large end of the probe into the adapter and then mount the adapter with the probe onto the printer. Make sure the probe is mounted properly and tightly.
- After each use, gently detach the probe from the adapter, clean the probe tip with DI water or 70% Ethanol, and dry with compressed air.
- Before reusing the probe, please properly sterilize the probe, especially the probe tip. The probe can be sterilized by UV light or 70% Ethanol.
- We don't recommend using the same probe for printing with different bioinks or by different users due to the risk of cross-contamination.



5.2. Multi-well plates

Our multi-well plates (24, 12, and 6-well) are compatible with our BIONOVA X™ 3D printer and printing probes. Our multi-well plates with high performance #1.5 cover glass (~0.170 mm) are suitable for high resolution imaging systems. The glass bottom is specially treated to enhance adhesion of the 3D printed tissues and avoid tissue detachment during medium change.

- Choose the appropriate bioprinting probe for different multi-well plate. For example, use the probe with a small tip for the 24-well plate, the probe with a medium tip for the 12-well plate, and the probe with a large tip for the 6-well plate.

- Our new multi-well plates are sterile and come in sterile plastic bags. The new multi-well plates can be used directly for bioprinting.
- To use the multi-well plate, remove the lid, add bioinks into the target wells and load the multi-well plate without the lid to the plate holder of the BIONOVA X^{TM} printer. Make sure the multi-well plate is mounted to the plate holder properly. Secure the multi-well plate with the clip on the plate holder.

  Warning: Inappropriate mounting of the multi-well plate may cause failure of the printing or even damage to the printer.
- After printing, carefully remove the multi-well plate from the plate holder of the printer.

5.3. Bioinks

Advanced BioMatrix provides a whole series of bioinks with various material properties. Please visit our website at www.advancedbiomatrix.com for details and application notes of the bioinks products.

Before printing on BIONOVA X^{TM} printer, load an appropriate amount of bioink solution to our multi-well plate. Please refer to the table in Section 4.3 for the maximum bioink load volume for each type of well plate. Overloading of the bioink solution will cause spills. Protect the bioink solution from light when it is not used in printing.

Make sure the bioink solution is sterile before mixing with cells for cell-laden structure printings.

We do not recommend reusing the excess bioink solutions left in the multi-well plate after bioprinting.