BIO X6: Operation and Maintenance Guide

1. Maintenance & Cleaning:

Surface Cleaning: Clean the surfaces with IPA (Isopropyl Alcohol). Do not spray IPA directly inside the BIO X6 chamber (to protect fans and electronics). Apply IPA to a cloth/tissue first, then wipe the surface.

Door Care: Do not use IPA on the front door (acrylic/polycarbonate), as it causes cracks/cloudiness. Use mild soap and water only.

UV Sterilization: (Standard procedure) Run the UV sterilization cycle before starting experiments to ensure a sterile environment.

HEPA Filtration: Always use the HEPA filter fan to maintain positive pressure and sterility inside the chamber.

2. New Print Procedure (Custom Design):

File Selection: Select **"Model",** Select - **"Surface/Well plate"** type.

Importing: Open you. gcode or .stl file from the **USB Pen drive**.

Proceed: Click **"Next"** to configure print settings.

3. Layer Height Configuration

Goal: Define resolution and adhesion properties.

Layer Height: Defines the resolution along the Z-axis.

Definition: If Layer Height is 1mm, the printer deposits a bioink layer exactly 1mm thick.

First Layer Height: Critical for adhesion and stability.

Higher First Layer (100–120%):

Use Case**:** Better for sticking to the surface; provides a stable base.

Material: Common for Hydrogels.

Note: Increasing height forces the printer to extrude more bioink to fill the thicker layer, which may cause high mechanical stress.

Lower First Layer (80–90%):

Use Case: Higher precision.

Material: Used for delicate cell-laden prints or when accurate pore geometries are required.

4. Advanced Printhead Strategies

Goal: Assign different materials to specific parts of the print.

Perimeter vs. Infill:

If you want to print the shell (perimeter) with one material and the inside (infill) with another, select: **"Divide Perimeter and Infill Printhead."**

Layer Groups:

If you want to print specific layers with different printheads (e.g., layers 1-10 with Head 1, layers 11-20 with Head 2), select: **"Create New Layer Group"** and assign the desired printhead to each group.

5.Structural Settings

Goal: Ensure print integrity and handle complex geometries.

Generate Support Structure:

The printer adds temporary material to hold up parts of the model that cannot be printed in "mid-air" (overhangs).

Close Seam Gaps:

The Problem: A small gap or hole can appear where the printer starts and finishes a layer loop. This is due to extrusion delays, pressure drops in the nozzle, or bioink elasticity/relaxation.

The Solution: Enabling **"Close Seam Gaps"** makes the printer slightly overlap or extend the extrusion at the seam.

Result: Adjusts the tool path so start and end lines connect properly, creating a more continuous and stronger structure.

6. Print Parameters & Environment

Goal: Fine-tune the physics of the printing process.

Printhead Check: Select and verify the correct printheads are mounted.

Nozzle Selection: Input the correct nozzle type/size (e.g., 22G, 27G) in the software.

Extrusion Settings:

Pressure: Set pneumatic pressure (kPa) based on viscosity.

Print Speed: Adjust translation speed (mm/s).

Temperature: Set nozzle and bed temperatures.

Flow Delay:

Adjusts the timing of bioink flow. It ensures the flow starts and stops at the exact right moment during printing to prevent oozing or dry starts.

Environment Controls:

Chamber Fan: Toggle On/Off.

Lights: Toggle On/Off and adjust intensity.

Photo crosslinking:

Light Source: Select wavelength (365nm, 405nm, 485nm, 520nm).

Focus: Ensure light is focused on the center of the print.

Travel Settings (Advanced): Configure non-printing movements to avoid collisions.

7. Calibration & Execution

Goal: Final alignment and starting the print.

Calibration:

Bed Levelling: Perform **“Automatic Bed Levelling”.**

Printhead Calibration: Perform Manual or Automatic calibration (following on-screen suggestions) to align the XYZ coordinates of all nozzles.

Start Print: Press **"Start Printing".**

Abort: You can Stop or Abort the printing process at any time if errors occur.

Note: Do not put hands inside the chamber while moving parts are active.

Droplet Printings:

1. **Printer Homing:** Navigate to **Printer Utilities** ---- **Home Move** --------- Select **Home**

   This resets the printer's axes to the zero position.

2. Select “Printhead Configuration”

- Attached Syringe printhead in printer

- Select the Syringe Printhead option in the software.

Retract Fully: Press "**Retract"** to move the plunger piston up to accommodate the size of your syringe.

Insert and fix the syringe into the printhead.

Engage Plunger: Press **"Extrude"** gently until the piston just touches the syringe plunger and a tiny drop of ink appears (priming the nozzle)

2. Droplet Protocol Design

Select Mode: Choose the "Droplet" printing mode.

Vessel Selection: Confirm the specific Well Plate type (based on the physical plate size/brand you are using).

Active Wells:

Select the specific wells you want to print into (e.g., A1, A2, B1...).

Assign the correct Printhead to the selected wells.

Print Order: Define the path the nozzle takes across the plate:

(a) Linear Horizontal: Prints row by row (left to right).

(b) Linear Vertical: Prints column by column (top to bottom).

(c) Serpentine Vertical: Prints down one column and up the next (snakelike), reducing travel time.

(d) Serpentine Horizontal: Prints across one row and back across the next.

4. Printing Parameters (Critical Settings) :

These settings control the volume and quality of the droplet.

Extrusion Rate: Controls the speed of the plunger movement.

Extrusion Volume: The exact total amount of bioink pushed out for one single droplet.

Retract Volume: The amount of bioink pulled back into the nozzle after dispensing.

Why it matters: Prevents the droplet from dripping while moving to the next well.

Extrusion Height: The distance between the nozzle tip and the bottom of the well during dispensing.

For Scaffolds/Practice Gel: Set to 90–100% of nozzle diameter.

For Cell-Laden Bioinks: Set to 80–95% of nozzle diameter.

Reason: A slightly lower height ensures the droplet touches the surface gently without dropping from a height, protecting cell viability.

5. Travel & Environment Settings

Travel Settings (Advanced):

Travel Height: How high the nozzle lifts while moving between wells. (Ensure this is high enough to clear the well walls).

Travel Speed: How fast the nozzle moves between points.

Environment Control:

Chamber Fan: ON/OFF (Turn ON for sterility).

Lights: ON/OFF (Adjust intensity as needed).

Photo crosslinking: Setup UV modules (365nm, 405nm, etc.) if your bioink requires curing immediately after dispensing.

Temperature: Set target temperatures for the Printhead (if heating/cooling is needed) and Bed.

6. Calibration & Execution

Initiate: Click "Go to Print".

Calibrate Printhead:

Select Automatic Bed Leveling.

Follow the prompt: Ensure the Magnetic Pin is straight.

Click Continue.

Important: After leveling is complete, always push down/retract the magnetic pin to avoid collision.

Manual Calibration (Z-Offset):

The machine may ask for manual verification, Fix the nozzle tip position over the first well (A1) or the designated calibration point.

Adjust the height carefully until the nozzle is at the correct dispensing distance (Extrusion Height).

Finalize:

Save Calibration.

Select **"Start Print.**

Once complete, the screen will display **"Finished".**