## High-Throughput Bioprinting of Spheroids for Scalable Tissue Fabrication

## **Supplementary Software**

## HITS-Bio

This supplementary software includes a LabVIEW program to operate HITS-Bio, controlling the motion axes (X-LSM200A, Zaber) and the solenoid I/O controller (750-881, WAGO), and monitoring the pressure sensor (3965, Adafruit) with an Arduino Uno board. Software compatibility was only verified for PCs with a Windows 10 and 11 64-bit operating systems. The software's compatibility was verified with the aforementioned hardware. SubVIs to control motion axes (<a href="https://www.zaber.com/software">https://www.zaber.com/software</a>) and Modbus communication via serial communications using TCP/IP for the solenoid valve control were integrated into the software, but future installation of them is not required to run this software. When the solenoid I/O controller is connected to your computer via an Ethernet cable, the Ethernet properties in TCP/IPv4 should be set to:

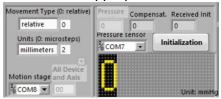
IP address: 192.168.1.1
Subnet mask: 255.255.255.0

To use this software for the operation of the HITS-Bio, the following modules are required:

- 1. LabVIEW Runtime (64-bit, 2019 SP1 f1 or higher, National Instruments)
- 2. NI-VISA drivers (Version. 19.5.0, National Instrument)

## Instruction:

- 1. Open the provided source file (Supplementary Software 'HITS-Bio\_Sortware\_2024') and save the files in the 'HITS-Bio-Software\_2024' folder on the PC hard drive.
- 2. Double-click on 'HITS-BIO software distr.exe' file.
- 3. Connect the appropriate COM numbers for the motion stage and pressure sensor.



4. Press 'Home All Axis" to set (0, 0, 0) positions for all axes.



Note: The step size can be controlled with pre-defined distance (0.01-20 mm) or customized values. Using the keyboard, the x-axis is moved with the  $\leftarrow$  and  $\rightarrow$  arrow keys, the y-axis with  $\downarrow$  and  $\uparrow$  arrow keys, and the z-axis with the 'Page Up' and 'Page Down' keys for moving up and down, respectively. Coordinates in the X, Y, and Z axes can also be set by entering numbers.



5. For the safety purpose and to protect samples and nozzles during operation, you can set the maximum Z position. This prevents the axis from going below the set position by selecting 'Set Max Z'. To use this function, activate it using 'ON' and 'OFF' buttons located under the 'Set Max Z' button.



- 6. Connect the WAGO I/O controller to the software using the "Connect" button.
- 7. For the initialization of the controller, press the 'Initialize Controller" button.



8. Main inlet pressure and aspiration pressure can be turned on/off using the 'Pressure' and 'Aspiration' buttons. The 'Minimum Vacuum' button can be used optionally to prevent the leakage of aspirated liquid during HITS-Bio operation.



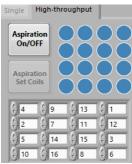
9. This HITS-Bio software allows control of single nozzle mode and high-throughput mode by selecting buttons the appropriate tap: 'single mode' and 'high-throughput mode'.



10. A total of 16 nozzles connected to the solenoid controller can be individually controlled using the buttons numbered '1' to '16.'



11. The individual nozzles in the DCNA can be defined and renumbered in order from 1-16 and controlled using 4x4 buttons.



12. During HITS-Bio operation, the predefined positions used can be saved in a .csv file for further experiments.



13. For automated motion, load a .csv file with X, Y, and Z coordinates and run it after selecting the automated motion speed and acceleration factor. Ensure the 'ON' button is selected. During automated motion, the motion can be stopped by switching the 'ON' button to the 'OFF' button.

