Operating Instructions

The syringe pumps are controlled by the 3D printer motherboard, which is already flashed with Marlin software and therefore works with G-code. There are two ways of controlling the pumps: a) using a computer connected to the board via USB and using direct control software such as PronterFace or Octoprint, with the latter able to be used remotely via network. B) writing the G-code file, uploading it on a microSD card and using the printer interface for printing the file without the need of a computer attached to the pumps.
A G-code file is a set of instruction which Marlin interprets line by line and passes the operations to the motors. The file is a simple text file saved as .gcode. For controlling the pumps, a few G-code commands are needed:

M92 X<Steps per mm> Y<Steps per mm> Z<Steps per mm>

M92 should be at the start of the G-code, as this command will set the number of steps required for moving one axis one millimeter, which in the case of the Ender 3 motors/drivers and the M5x0.8 mm threaded rod is 4000 steps per mm. The Ender 3 uses stepper motors with a step angle of 1.8 °, or 200 steps per revolution. The TSMC2208 stepper drivers are set to 1/16 microsteps, which is the default setting for the Ender 3. The pitch of the leadscrew is 0.8 mm. This gives the value of 4000 steps per mm. However, as we are dealing with flows, we found that it is more user-friendly to change this command from steps per mm to steps per mL. This can be easily done by calculating the distance required to push a volume of 1 mL depending on the diameter of the syringe used. To do this, the inner diameter (I.D.) of the syringe needs to be determined. Next, the area of the plunger can be calculated. The linear syringe travel to deposit 1 mL can be calculated by dividing 1 cm^3 by the plunger area (cm^2). Divide this number by the steps per mm value (4000) to get the steps per mL. We have attached a simple calculator in the supplementary material to calculate the steps per mL using the diameter of a syringe as input. As an example, a 10 mL syringe has a diameter of 15.6 mm and the calculated steps required to move 1 mL are 20927, a 5 mL syringe has a diameter of 12.3 mm and the calculated steps are 33663.
Another thing to take note of, is that in the normal configuration, the X and Y motors are inverted, so this should also be set in the M92 command by giving negative step/mm values. A M92 set up using two 5 mL syringes on the X and Z axes and a 10 mL syringe on the Y is therefore:

M92 X-33663 Y-20927 Z33663
This will set the number of steps per mL in such a way that the rest of the G-code will use this to calculate the pump movements.

M302 S0
This command will set the printer to move the motors without checking the temperature of the hot end. This is usually a 3D printer safety check for avoiding extruding material when there are some problems with the hot end. In this case the hot end is detached, so there is no risk of thermal runaway.

M211 S0
This command will disable the endstops which may cause problems as they are not connected.

G91
Use relative positioning. This is important when multiple movements are used, otherwise the motors will start from a 0.0.0 position and use that as reference point.

G4 S<time in seconds>
Pause the commands for a set amount of time.

G1 X<mL> Y<mL> Z<mL> F<mL/min>
This is the command to move the syringe pumps at certain flowrates. As we set up the steps/mL in the M92 command, now all the G1 codes are in mL/min. This can be a positive number or a negative number, positive for pushing and negative for retracting.

For example:
G1 X0.5 F1
Will use syringe pump X and will deliver 0.5 mL of liquid at a speed of 1 mL per minute.
Multiple syringes can be used at the same time:
G1 X0.5 Y0.5 Z0.5 F1
Will deliver, at the same time, 0.5 mL of liquid from the pumps on X, Y, and Z at a speed of 1 mL/min
For using different flow speed for different pumps, the total amount of liquid should be calculated taking in account that all the pumps will reach the end at the same time. For example:
G1 X2 Y1 Z2 F1
Will deliver the set amount of liquid at different flow speed, using 1ml/min on Y, and 2mL/min on X and Z.
For commenting on G-code files the ";" character should be used. This can be useful in commenting the G-code with metadata.

An example of full G-code (VideoS1):

```
; Experiment 01 20/05/2021
; 10 mL syringes on the three pumps
M92 X-20927 Y-20927 Z20927 ; set the proper steps/mL for the 10mL syringe
M302 S0 ; print without checking temperature
G91 ; relative positioning
; deliver 1mL at 2mL/min sequentially
G1 X1 F2
G1 Y1 F2
G1 Z1 F2
; wait for 10 seconds
G4 S10
; deliver 1mL on X and Z at 1mL/min, 2mL on Y at 2mL/min
G1 X1 Y2 Z1 F1
; wait 10 seconds
G4 S10
; deliver 1mL at 2mL/min sequentially
G1 Z1 F2
G1 Y1 F2
G1 X1 F2
```

Without previous knowledge in programming and in a few lines of G-code it is possible to program the syringe pumps to do multiple operations. This file can now be saved as .gcode,

uploaded on a microSD card, and run on the Ender 3 syringe pumps. Or it can be sent using a computer connected to the USB.
G-code files can be easily shared between labs for improving reproducibility of experiments.