# Waffle Assembly Instructions

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## 1 Introduction

This document details the materials and steps required to manufacture and assemble the WAFFLE platform, as presented in the 'WAFFLE - An automated IoT-enabled platform for enhancing the performance of electrochemical biosensors at research level' paper. The 3D printed parts shown were manufactured using a Creality Ender 3 V3 KE printer with PLA filament, but any printer with a standard bed size (23.5 x 23.5 cm) should be suitable. Likewise, we use a 50 W Epilog mini laser cutter and Cricut Maker 3 plotter cutter for making the fluidic devices but any laser cutter capable of cutting through 3 mm PMMA and any plotter cutter should be suitable for this purpose.


**Figure 1:** The Waffle platform

**WARNING - High voltage** The mp-6 micropump operates at high voltage which can pose a serious threat to life. The same applies to the valves which require high current to open. Extreme caution must be exercised when handling those components and their drivers. Please read through the safety datasheets of these components before attempting to build and operate this system on your own.

# 2 Materials

Begin by gathering all the required materials (an exhaustive list is provided below).

## 2.1 Harware

- 3D printed device enclosure (fluidic and electronic chambers and lids), electrode connector, frames for mp-6 pump, flow sensor and mp-damper - Figure 2.
- PCBA - custom design and assembly ordered from JLCPCB (circuit schematic can be seen in the Appendix - A1; Gerber files, BOM and pick and place files for shift register, diodes, resistors, capacitors and MOSFETs available on GitHub page)
- ESP32-S2-MINI-2 by Espressif (DigiKey)
- mp-Lowdriver Pump Driver (Darwin microfluidics)
- mp-valvedriverM and mp-valvedriverT for memetis and Takasago valves respectively (Darwin microfluidics)
- Molex 12 positions 1.25 mm pitch right angle connector (DigiKey; for pump connection)
- Pin Header, Right Angle, Signal, 1.25 mm, 1 Rows, 6 Contacts, Surface Mount Right Angle (DigiKey; for flow sensor connection)
- Standard picoblade-to-picoblade cable 15134-06XX series, 6 pin (Digikey, for flow sensor connection)
- Breakaway header pin rows for soldering
- mp-6 liq micropump (Bartels Mikrotechnik)
- mp-damper (Bartels Mikrotechnik)
- SLF3S-1300F flow sensor (Sensirion)
- NC microvalves, 2 x M1.6 bolts per valve and cables (memetis)
- USB A to micro USB B (Amazon)
- Steel comp spring,26L x 11mm diameter (RS Components, for electrode connector)
- 8-pin spring contact header (RS Components, for electrode connector)
- Electrical wire
- 18-way IC socket (RS Components, for connecting the mp-lowdriver)
- M3 screws for securing the PCB for the base

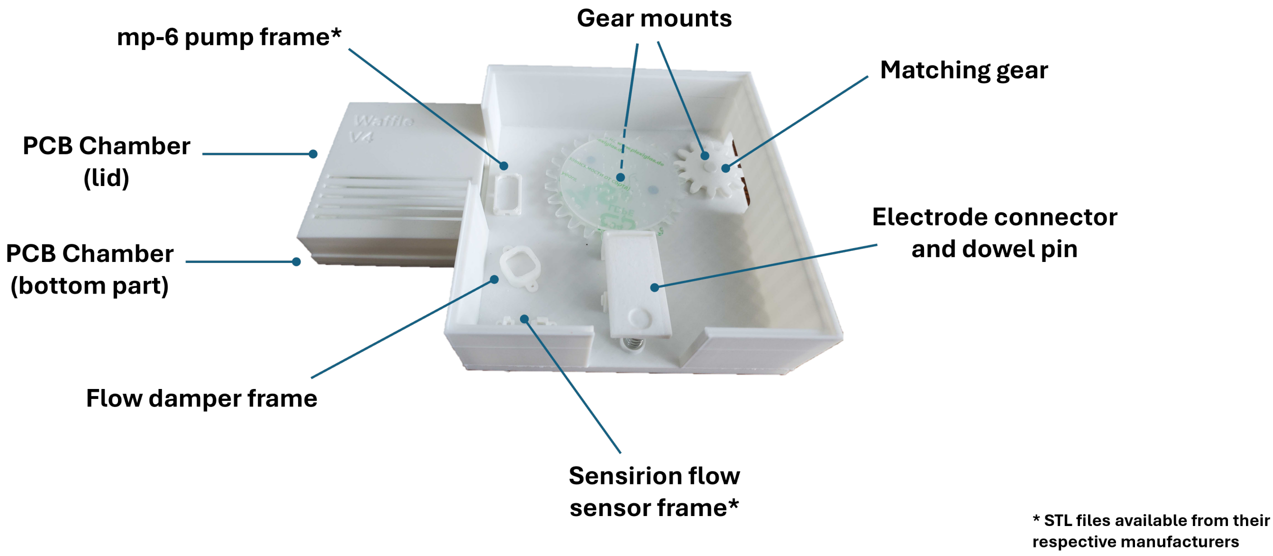



**Figure 2:** Parts to be 3D printed for assembly

## 2.2 Fluidic cartridge

- Mini luer fluidic connectors (male and female) - Fluidic 331 and Fluidic 631 from microfluidic ChipShop.
- Silicone tubing for mp-6 pump (Bartels Mikrotechnik)
- 0.5 and/or 4.5 ml reservoirs with luer interface - Fluidic 387 and Fluidic 232 from microfluidic ChipShop.
- Luer to mini luer fluidic adaptors - Fluidic 390 from microfluidic ChipShop.
- 3 mm thick PMMA sheets (Stockline Plastics Ltd or similar provider)
- 0.1 mm thick PET spacer - Amazon
- Adhesive transfer tape (3M™ 468MP) - Amazon
- Waste reservoir e.g., 15 ml tube

## 2.3 Equipment

- Soldering iron, solder wire and flux paste
- Laser cutter/engraver (we use a 50 W Epilog mini)
- Cricut Maker 3 plotter cutter
- 3D printer (we use a Creality Ender 3 V3 KE)

# 3 Manufacturing and assembly

## 3.1 3D printed parts

1. First download the STL files from the Github page for the parts listed in Figure 2. The frames for the flow sensor and mp-6 pumps may be found on the respective manufacturer's website (Bartels Mikrotechnik and Sensirion).

2. Open the preferred slicer application for the 3D printer used (we use Creality Print 5.0) and import the desired model

3. Set printing settings (print flat without supports, set nozzle size e.g., 0.4 mm, layer height e.g., 0.2 mm, 15% grid infill, 0.8 mm shell thickness, monotonic surface pattern; set material e.g., hyper-PLA)

4. Print the components and gently remove from the PEI print plate.

5. If printing the electronic and fluidic enclosures separately (due to size limitation of 3D printing bed), glue these together using loctite superglue as shown in Figure 2.

## 3.2 PCBA

1. Order the PCB with pick and place assembly from JLCPBC or similar PCB manufacturer using the Gerber files, BOM and pick-and-place file provided on the Github page. Figure 3 shows the latest version of the PCBA that you should receive.

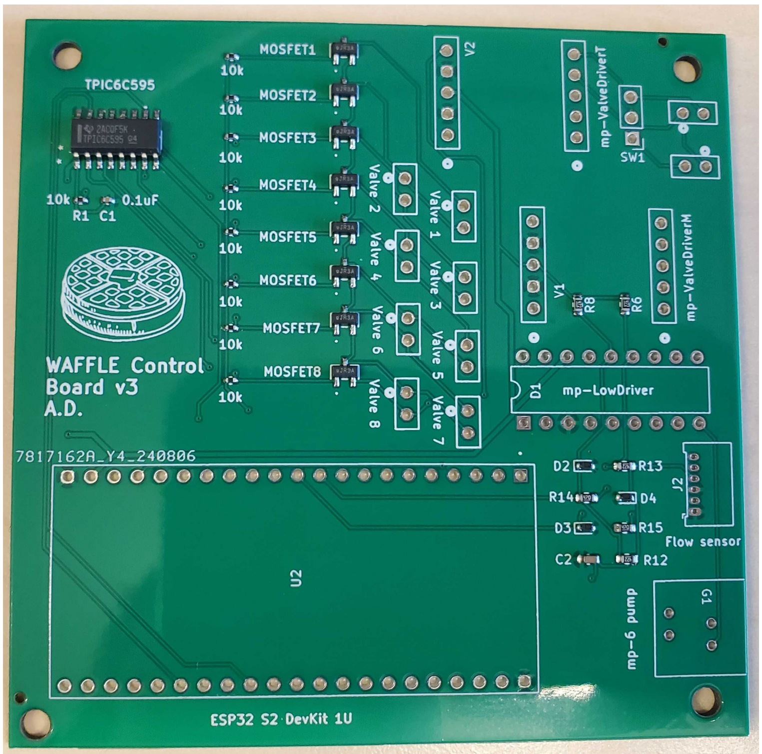



**Figure 3:** WAFFLE PCBA - as received

2. Begin by making 2 rows of 21 contacts for connecting the ESP32 microcontroller to the board, 4 rows of 5 contacts for the two valve drivers and 8 rows of 2 contacts for the valve wires using the breakaway header pins.

3. Solder these, the 18-way IC socket and connectors for the micropump and flow sensor to the PCB.

4. Install the ESP32 microcontroller, pump and valve drivers to the soldered pins taking care to observe correct orientation of the components. Datasheets of these drivers and their pinout may be found on the manufacturer's website.

5. The final assembled PCB should look as shown in Figure 4.

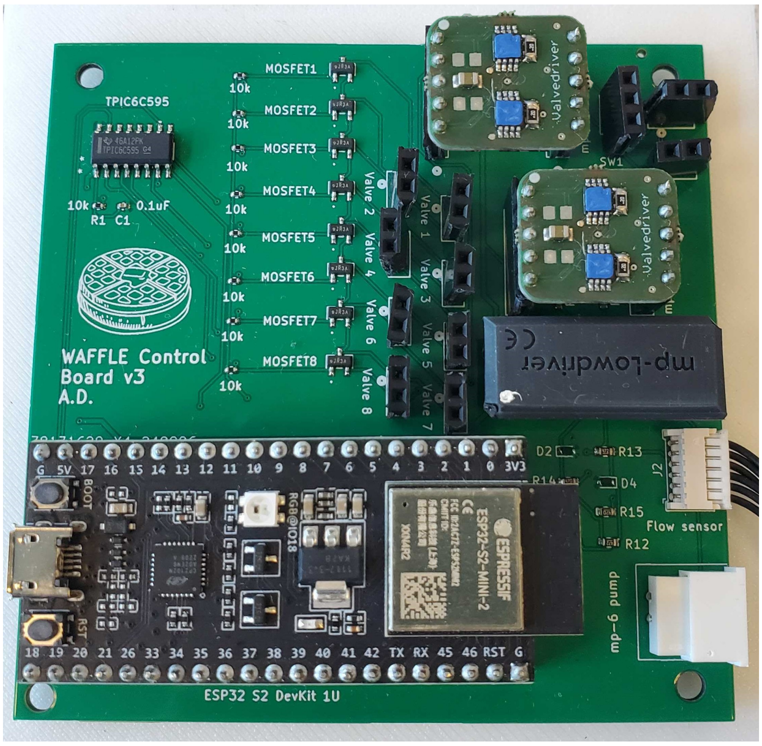



**Figure 4:** WAFFLE Fully assembled PCBA

## 3.3 Fluidic device

The fluidic cartridge consists of the layers shown in Figure 5, namely 3 layers of laser cut 3 mm thick PMMA and 2 layers consisting of double sided tape with a PET spacer in the middle.

1. If a laser cutter is available, download the .dwg files from the Github page and cut the inlets, middle and outlets layer using suitable power and speed settings recommended for 3 mm acrylic. Since we use a 50 W Epilog mini laser we use: power = 100%, speed = 18%, frequency= 5000 Hz (these values may need to be adapted based on the laser machine used).

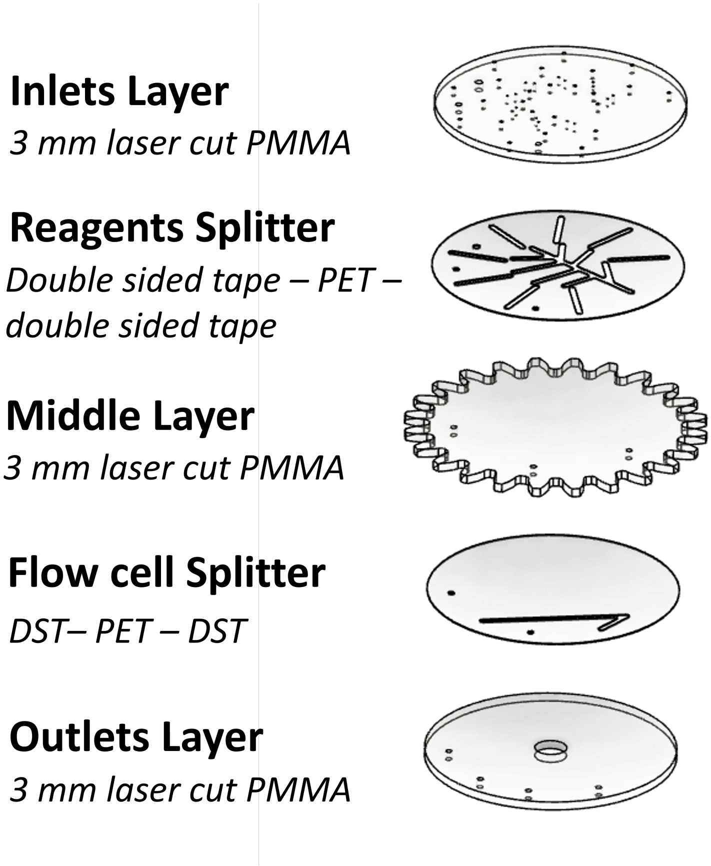



**Figure 5:** Layers of the reagent splitter

2. Carefully install the double sided adhesive onto both sides of the PET spacer using an adhesive roller ensuring no air bubbles are trapped. A spacer is used in this case for ease of handling as the adhesive we use is very thin. To skip this step, you can use an adhesive with an in-built PET spacer such as the 3M™ Microfluidic Diagnostic Tape, 9972A (a free roll can be requested from Solventum).

3. Place the adhesive on the cutting mat (we use the Cricut Standard Grip), flatten if needed using an adhesive roller. If the edges of the adhesive are raised, stick them to the mat using scotch tape.

4. We use Cricut design space for controlling the plotter cutter. The cutting files for the adhesive layers may be found using the following link.

5. Open the Cricut design space and click 'Make'. Set the material to 'Adhesive - Double Sided', insert the cutting mat into the cutter and start cutting.

> **Info:** In case you may not have access to a laser or a plotter cutter, we can manufacture both of these layers and send it to you. If that is of interest please get in touch using this link or writing to us at biomed-shed@strath.ac.uk.

6. Figure 6 provides a reference map of all the through holes and their meaning. Carefully install the 'reagent splitter' adhesive onto the inlets layer making sure

that all the inlets to the reservoirs and valves are aligned and unobstructed. Use the through holes as installation guides. Once installed, go over it a few times with the adhesive roller to make sure no air bubbles have been trapped.

7. Repeat this process with the 'flow splitter' adhesive and finally attach the 3 PMMA layers together. If using a pressure sensitive adhesive (e.g., 3M™ 9972A) apply consistent weight to the assembled device for a few hours or overnight before use to ensure the adhesive is at full strength.

8. Glue the mini luer adaptors using Loctite All Platics superglue to the inlets (R1 - R8) and outlets (O1 and O2) of the device. (NOTE: using an adhesive specifically formulated for polypropylene - a low surface energy plastic - is important to ensure good attachment).

9. Install the valves into the valve inlets using the M1.6 bolts ensuring the o-rings are adequately compressed. If needed, lubricate the o-rings using silicone grease (RS Stock No.: 494-124) for better sealing performance.

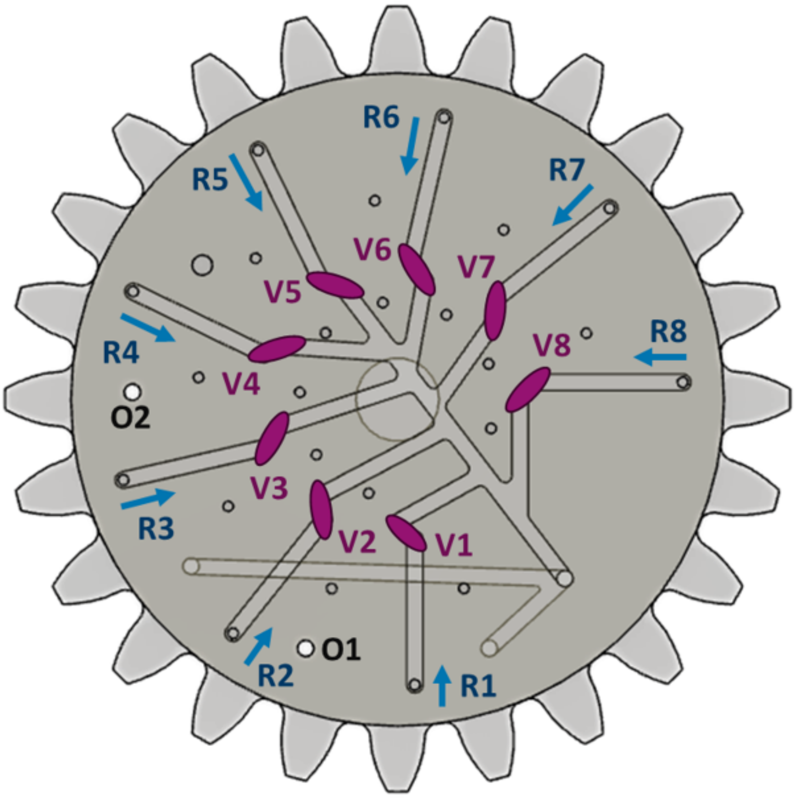



**Figure 6:** Through holes on the reagent splitter and what they correspond to

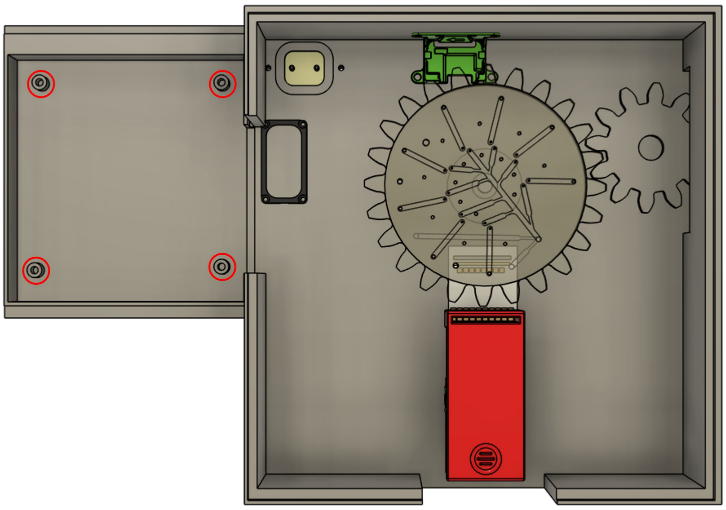
10. Cut the desired electrode channel to fit your electrode using the Cricut and the same double sided adhesive as the other channel layers. We use the same channel geometry as have described in a previous publication as we used the same biosensor design.

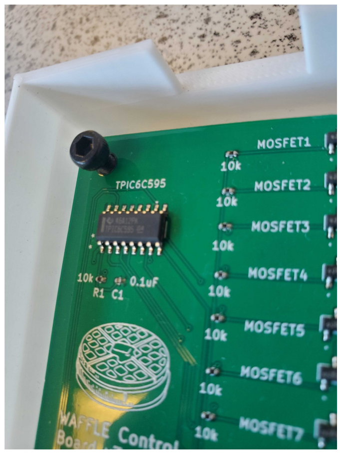
11. Attach the channel layer to the electrode using a similar process to that shown in the Supplementary video of our previous publication.

12. Install the electrode/flow chamber onto the bottom side of the cartridge so that the outlet coincides with O1 or O2 and the inlet coincides with one of the two oulets of the flow cell splitter. If using only one electrode/flow chamber seal the unused outlet using tape.

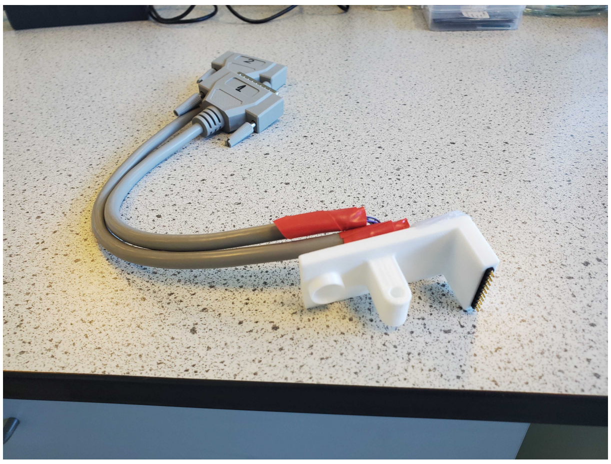
## 3.4 Device assembly

1. Attach the PCB onto the installation bosses (marked with red circles) on the bottom of the PCB chamber using the M3 bolts. The screws should create a thread as they insert into the plastic.

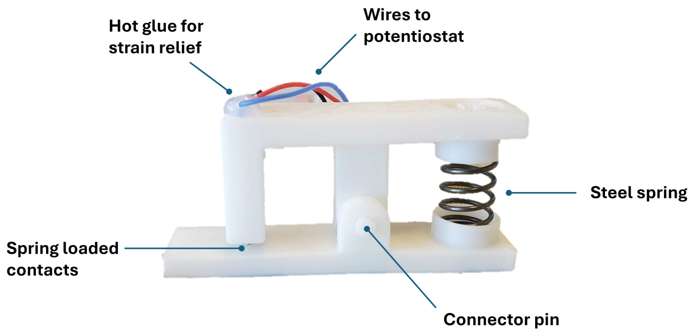




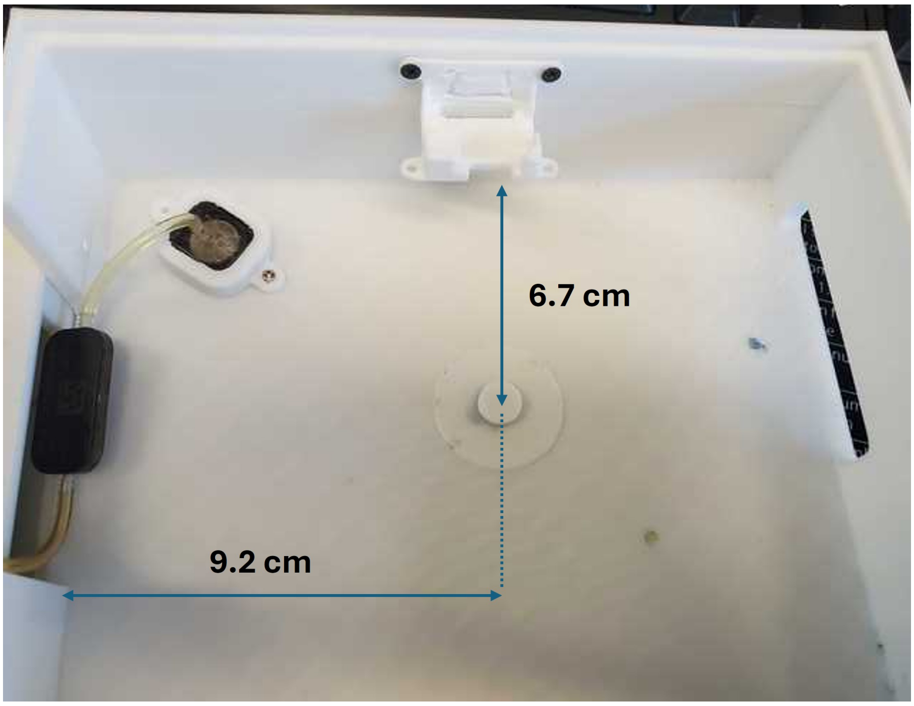

2. For making the electrode connector, solder the electrical wire to the rigid end of the spring loaded pins. Carefully thread the wire through the body of the connector top and click in space. To provide additional strain relief on the solder joints you may wish to use a hot glue gun to keep the wires in place. In the figure below we solder the spring connectors straight to the multiplexer unit cables.



3. Place the steel spring inbetween the base of the fluidic chamber (in its installation guide) and the top of the connector and secure it under tension by threading the dowel pin in place. The connector part should look as shown below.




4. Next, glue the reagent carrousel mounting part to the bottom of the fluidic chamber roughly in the location shown below. If using a different reagent carousel design, adjust the location until the pins of the electrode are well aligned with the spring contact pins of the electrode connector, then glue the mounting attachment into place.




5. Bolt in the flow sensor and damper frames roughly in the positions shown in the previous figure.

6. Install the damper, pump, flow sensor and reagent carousel into place and connect the components using silicone tubing as shown below. Make sure a suitable waste reservoir is added at the outlet of the flow sensor to capture the dispensed fluid.


7. If using the valves, connect the valve pins to the valve header pins labelled 'Valve 1-8' on the PCBA.

## 3.5 Software

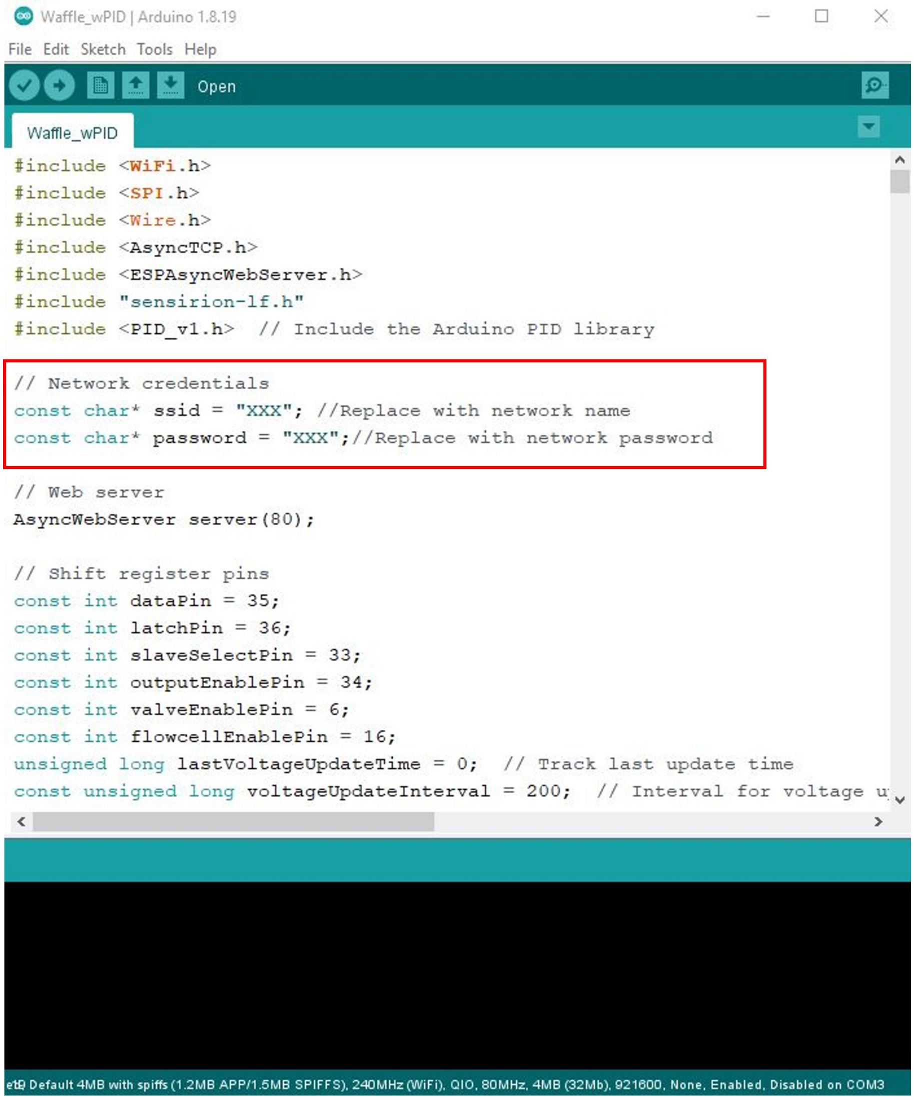
1. Download the Waffle_wPID.ino arduino sketch from the Github folder. Before opening make sure you have the Arduino Legacy IDE (1.8.19) installed on your computer.

2. Open the Waffle_wPID.ino file and update the ssid and password to the network (WiFi or hotspot) available.

3. Compile the sketch and ensure no errors pop up (you may need to download the esp32 library from Espressif Systems and then set the board in the Tools tab to ESP32S2 Dev Module and select the correct port).

4. Connect the laptop to the ESP32 microcontroller via the USB-to-microUSB-B and upload the sketch. Allow a few minutes for the sketch to upload.

5. Once the sketch has been uploaded, open Serial Monitor and adjust the baud rate to 115200 and deselect autoscroll.




6. The microcontroller will first attempt to connect to WiFi. Once connection is established, the IP address of the microcontroller should be appear in the command window. Copy pasting that into your preferred web browser will bring up the GUI where you can set the desired flow rate setpoint, enable or disable the micropump and trigger the desired valves.

7. To trigger the pump, input the desired point flow rate setpoint and tick the 'Enable lowdriver' box. Upon doing this, a live feed of the pump voltage, flow rate determined by the flow sensor and setpoint flow rate will be displayed in the IDE command window.

> **Info:** If the flow reading does not correspond to the actual volume of liquid dispensed by the system you may need to perform a flow sensor calibration e.g. using a syringe pump and dispensing known volumes/flow rates and measuring the dispensed output with a scale. Fitting a linear equation through that data (flow sensor reading/actual flow rate) should help you scale the flow sensor reading to the actual flow rate. Update the equation present in the code to reflect that.

8. To trigger the desired valve, simply click on the green 'Enable' button below each valve label.

> **Info:** The system was designed to only trigger maximum 2 valves simultaneously while the rest are maintained in an 'off' state. If more valves need to be triggered at the same time, the system will have to be updated to provide additional current handling capabilities. Care must be taken to not damage the components.

9. The system is now ready to use!

# 4 Appendix