**Arduino MQTT code for microfluidic system**

No sensor for fluid front so everything is done via trusting stepper is accurate and fluid isn’t slipping within pump head. I need to quantify several key quantities to make this work.

1. How long for fluid front to get from multiplexer to chamber
2. Amount of fluid chamber holds
3. Diffusion will happen within the line. How big are diffusion zones?
4. Fluid flow as a function of stepper speed

How to determine these things?

Key aspect in much of these experiments is green food colored liquid and clear liquid. One vial is full of green water and another clear water.

1. Load green liquid just into multiplexer. Flush entire line with clear. Run green through entire line and pour liquid into different Eppendorf every 3 seconds. Look at OD at 625nm on nanodrop to find green [C] as a function of time.
2. Pump fluid into chamber in 1 second bursts followed by 2 seconds of rest. Once fluid rises into drain tube, the chamber is filled and we know how many seconds it took and the flow rate.
3. Pump exactly 100uL of green fluid into main line and switch to clear for the rest of the time. Sample into eppendorfs every 3 seconds. Check OD at 625nm for green [C].
4. Run pump for 30 seconds and capture all liquid in eppendorf. Then weight Eppendorf and convert to volume. Make range of step speeds between 1-9ms delay between steps.

How to interpret

1. Run experiment 4 first. Plot and find linear region. This is important because we need repeatable actions. Peristaltic pumps are approximately constant flow devices, but as back pressure approaches max pressure the system can handle, it slows down. The approximation is only valid in low pressure regimes. If we are in a linear area, we are then in an area where that approximation is true.
2. Run experiment 3. Going from 0 OD to the plateau region and back down to zero can define the transition zone size at the speed we are running.
3. Run experiment 1. Since we know the transition zone size, we can sub that from the green front to find the ‘real’ front and then find out the volume of the main line and how long it needs to be pumped from multiplexer to line end.
4. Run experiment 2. Nothing else is needed but to use the flow rate to solve for volume.

Findings

1. 18 seconds from multiplexer to line end
2. 7ms steps is within pressure limits of chamber and in linear region
3. 7ms steps has 660uL/min flow rates.
4. Chamber holds about 60uL (5-6 seconds to fill).

Code

To code this system, I am using a MQTT command structure to send commands to the Arduino and get data back (nothing with data back is currently used though).

**Functions needed:**

1. Auto\_Load(time\_small, time\_large). Load in all 8 liquids into multiplexer. Numbers 2-7 just reach multiplexer while 1 and 8 flow through more. Time\_small is time to pump liquid from Eppendorf to multiplexer given a speed of 7ms per step. Time\_large is time to load larger volume liquids of 1 and 8 into multiplexer. Will make default for both.
2. Dispense(liquid selection, volume, plex\_chamber\_time). Moves volume defined of liquid selected into chamber. Plex chamber time is time to flow from multiplexer to chambers end. Plex chamber time is a default answer. Acts different if volume requested is larger than volume from multiplxer through chamber. Difference is PBS flow is not activated if volume is larger, but is if not.
3. Flow(liquid selection, time, chamber\_volume, plex\_chamber\_time). Flow liquid selected through chamber. Defaults include time, chamber\_volume and plex\_chamber\_time. If defaults are used, 4x chamber volume is used. If time is used, it overrides the last two parameters of chamber\_volume and plex\_chamber\_time.
4. Bleach(time). Flows bleach solution into chamber and keeps it on for time amount of time. Time will be default. Uses dispense as backbone.
5. stain(time). Flows stain solution into chamber and keeps it on for time amount of time. Time will be default. Uses dispense as backbone.
6. Water\_fill(fill or drain, time). Aquarium pumps to fill or drain outer chamber with water. Default is time at max speed. Fill or drain will be string type entries.
7. Nuc\_touch\_up(time). Flows hoescht solution into chamber and keeps it on for time amount of time. Time will be default. Uses dispense as backbone.