

Supplemental Information

R_2 / R_1	Max ΔP to achieve stream selection	Functionality
1.8	0.4	Stage 1 too sensitive
7.8	0.6	Good
13	1.8	Vortices occurring at Stage 1

R_3 / R_2	Primary stream refresh time (ms)	Secondary stream refresh time (ms)	Functionality
0.03	55	200	Stage 2 too sensitive
0.06	70	140	Switching frequency possible up to 4 Hz
0.1	130	450	Switching frequency possible up to 2 Hz

Device characteristics

R_1	6.9 E+12	kg/(m ⁴ /s)
R_2	5.3 E+13	kg/(m ⁴ /s)
R_3	3.2 E+12	kg/(m ⁴ /s)
Upstream outlet flow rate	840	μL/min
Downstream outlet flow rate	70	μL/min
Velocity at worm's nose	55	mm/s
Flow rate at worm's nose	35	μL/min
Switching time	60	ms

Table S.1: Influence of the ratio of resistances R_2/R_1 over stream selection at Stage 1. “ ΔP to achieve stream selection” refers to the maximum difference in pressure between all inlets leading to selection chemicals.

Table S.2: Influence of the ratio of resistances R_3/R_2 over stream selection at Stage 2 and switching frequency. Primary streams refers to the stream pushed over the worm's nose and secondary stream refers to the stream diverted away from the worm's nose.

Table S.3: Device characteristics. Resistance values R_1 , R_2 , and R_3 were calculated based on the channel geometry. All other parameters were measured while operating the device with inlet pressures around 5 psi.

Supplementary Video 1: Video showing delivery of a 2Hz stimulus sequence to an immobilized worm. Stimulus streams are labelled with food dyes. The field of view is located at Stage 2 of the device, with the upper stages and inlets located in the downward direction.

Supplementary Video 2: Video of a single animal from Fig. 6 experiment demonstrating ASH neuronal activity to repetitive stimulation of either 1M glycerol or .5M NaCl. Each stimulus pulse duration is 10s. The leftmost panel shows an immobilized worm with its nose pointed towards the right into the stimulus channel. The upper-right panel displays the neuronal activity of the ASH neuron over time. The bottom-right panel displays the driving stimulus signal as quantified by average fluorescence intensity at the worm's nose. Video playback is sped up 5x.

System Design and Assembly

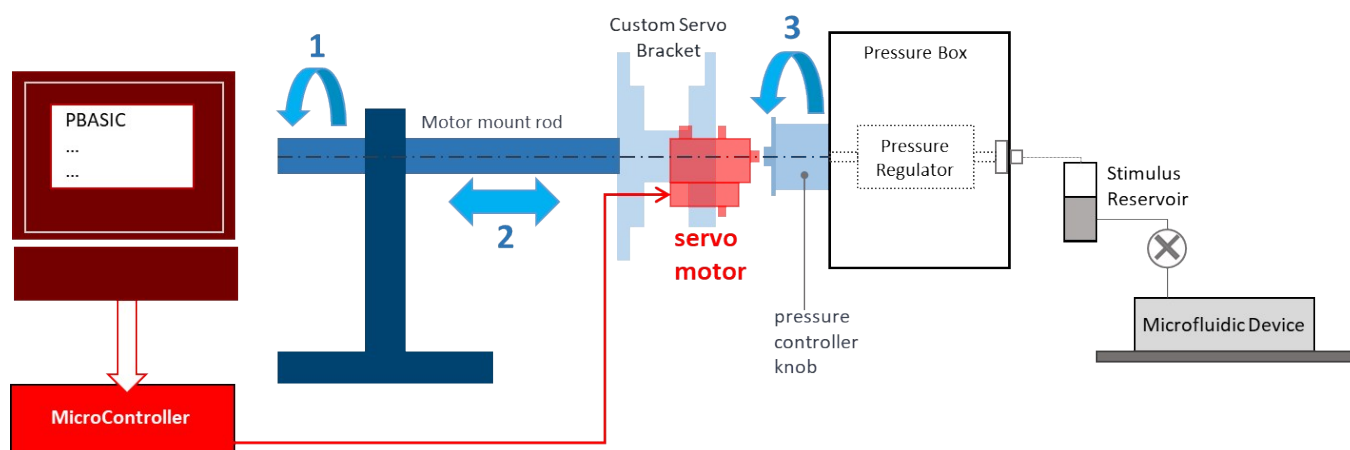


Figure SI 1: Schematics of the experimental system for automating one line of pressure control.

List of components to set up the pressure control of the platform:

- Computer software (BASIC Stamp Editor Software)
- Microcontroller (BASIC Stamp II Educational Board, Parallax Inc.)
- Servo motor (LS-0009AF, Metal Gear Digital) x4
- Motor mount (custom-made) x4
- Pressure-box

Assembly: Servo horn was glued to the regulator knob of the pressure box. Motor was aligned to fit to the horn via the custom servo mount with rotational and translational freedom (arrows 1 and 2 on Fig. SI1). To achieve rapid, automated pressure regulation, servo motors were mounted axially on optical assembly rods, and the entire motor assembly could be rapidly and reversibly engaged and disengaged to the pressure regulator knob, allowing for manual alignment (arrow 3 on Fig. SI1). After which, commands sent via PC to the microcontroller enable rapid and precise rotations of the servo motor to adjust the rotation of the regulator knob.

The resulting platform design is generic and fairly cheap. Various types of servo motors, microcontrollers, pressure regulators, and mounting methods can be substituted to achieve a working, in-house pressure control design to work with the microfluidic device. Also, commercial products that offer flow control for pressure-driven flow in microfluidics should work with the design.

System Calibration and Imaging Workflow: Calibration starts with adjusting manually the pressure so that the stimulus streams contribute equally to the total flow fraction. Then, servo motors are set to the home position using commands via the microcontroller (the servo motors used here, chosen for speed, rotate ± 45 degrees from the home position). Finally, the servo motors are locked to the pressure knobs. Calibration is only needed for initial setup. Tens of animals can be successively assayed without requiring any re-calibration.